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STUDIES IN EXPERIMENTAL GLYCOSURIA

IX. The Level of the Blood-Sugar in the Dog Under Laboratory Conditions

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It is a well-established fact that the anaesthetizing and preparation of laboratory animals causes a varying and often very considerable degree of hyperglycaemia to develop. Although there can be no doubt that the influence of certain factors on the blood-sugar level will be masked by the presence of this condition, yet it has so far been impossible to obviate it by procedures which are not open to objections that are probably as serious as the presence of the excess of sugar in the blood.

Both E. L. Scott (1) and Shaffer (2) have recently shown that the blood-sugar level in the dog and cat is really much below that usually taken as the 'normal' in experiments bearing on sugar metabolism. Scott concludes that "if consistent results are to be expected, the animals (cats) must be . . . . killed without pain or excitement," and Shaffer, working on the dog, shows that the removal of blood from a vein with entire avoidance of any excitement on the part of the animal, yields blood-sugar values which are about one-half those which have been considered by previous workers as normal. Both investigators emphasize the diabetic influence of the excitement caused by giving a general anaesthetic, and in a later communication, Shaffer (along with Hubbard (3)) shows that the initial hyperglycaemia may be avoided, or, if present, caused almost to disappear by forced respiration through a tracheal cannula.

Similar observations by other investigators are referred to fully in the above papers.

If we are to confine our investigations on the behaviour of the sugar level of the blood to unanaesthetized animals, however, a very limited field will be open to us, for we shall be unable to study many of the factors which influence the glycogenic functions of the liver. For purposes of following the blood-sugar level from day to day, the precautions advocated by the above workers must henceforth be strictly adhered to, but for the large group of observations requiring surgical interference, and therefore anaesthesia, an entirely different method must be followed in obtaining normal standards with which to compare the experimental results. Shaffer and Hubbard suggest that in such cases the initial hyperglycaemia, since it is largely due to dyspnoea, should be avoided by forced artificial respiration. One of us (J. J. R. M.) (4) some years ago employed this method to avoid the dyspnoeic hyperglycaemia brought about in dogs by stimulation of the central end of the vagus nerve. It was also used in experiments involving stimulation of the splanchnic nerve. The forced ventilation was always found to cause a decline in blood-sugar, but the method was abandoned as a routine practice in subsequent work because it was concluded that the depression in the CO_2-tension of the blood involved in using it would introduce conditions which were at least as unphysiological as those due to a slight excess of sugar. We have frequently reconsidered the advisability of using forced respiration or insufflation of oxygen in our experiments, but as work has accumulated showing, on the one hand, the close relationship between the CO_2-tension of the blood and its reaction and, on the other, the susceptibility of the physiological activities of nerve centers, as well as of many organs and tissues—including almost certainly the glycogenic function of the liver—to the reaction of the blood, we have abandoned the idea.

These difficulties in securing constantly low 'normal' values for blood-sugar in laboratory experiments make it necessary to adopt other standards with which to compare the results obtained during some experimental procedures. The ideal being unattainable at present, there remains available one of two methods, either: (1) to determine the blood-sugar level of each animal for some time before bringing about any experimental change, or (2) to use, for comparison with the experimental animal, data secured from a sufficient number of anaesthetized animals in which all the conditions are as similar as possible to those obtaining in the experimental animal.

We have hitherto employed the former of these methods although, as already stated, we have recognized the limitations due to the fact that the initial disturbances dependent upon etherization, etc., might mask many of the results. For further work which we contemplate, however, it will be necessary to employ the second method, and some of the data which we have collected to this end are given in the present paper.

Methods. Dogs were employed in all the experiments. Most of these were removed from the stock kennels several days before being used and were either starved or given a liberal diet of bread broken up in a meat broth. Certain of the animals were given 5–7 grams of cane sugar per kg. body weight, dissolved in water, by stomach tube, on the evening preceding the experiment. Etherization was brought about as quickly as possible, and the animal was then tied out on a warmed operating table and a tracheal cannula introduced and connected with an anaesthetic bottle. Throughout the remainder of the experiment the concentration of ether in the inspired air was kept as constant as possible. The arterial blood pressure, the respiration and the rectal temperature were carefully watched.

After opening the abdomen the aorta was ligated between the coeliac axis and renal arteries, and the vena cava tied at the same level. A cannula was then introduced in the vena cava so that its open end lay opposite the hepatic veins. This cannula was plugged by a pipe cleaner. To secure samples of blood from the portal vein a similar cannula was inserted in the pancreatico-duodenal vein with its free end just at the vena porta. For the analyses samples of blood were removed, usually at intervals of two minutes, by removing the pipe cleaners and connecting a pipette to the cannula. A sufficient amount of blood was removed to be certain that the cannula was filled with that present in the particular vein under observation. This pipette was then removed and a moistened 2 cc. pipette connected with the cannula. There was practically never any trouble with clotting, for if a clot did form it remained adherent to the cleaners and was withdrawn with them.

The 2 cc. samples of blood were immediately transferred to test tubes containing 8 cc. water, and the sugar content was ascertained by one of the methods described elsewhere by one of us (R. G. P.) (5).

Portions of liver were also removed at the end of the experiments for glycogen estimation.

Results. The following features of the blood-sugar level have occupied our attention in the present investigation, viz: (1) the extent of the fluctuations occurring under normal conditions; (2) the initial height of

the level in relationship to the amount of glycogen in the liver; (3) the relationship between the levels in the blood of the portal vein and vena cava.

Although we have performed a considerable number of experiments, we do not consider that we have by any means sufficient data from which

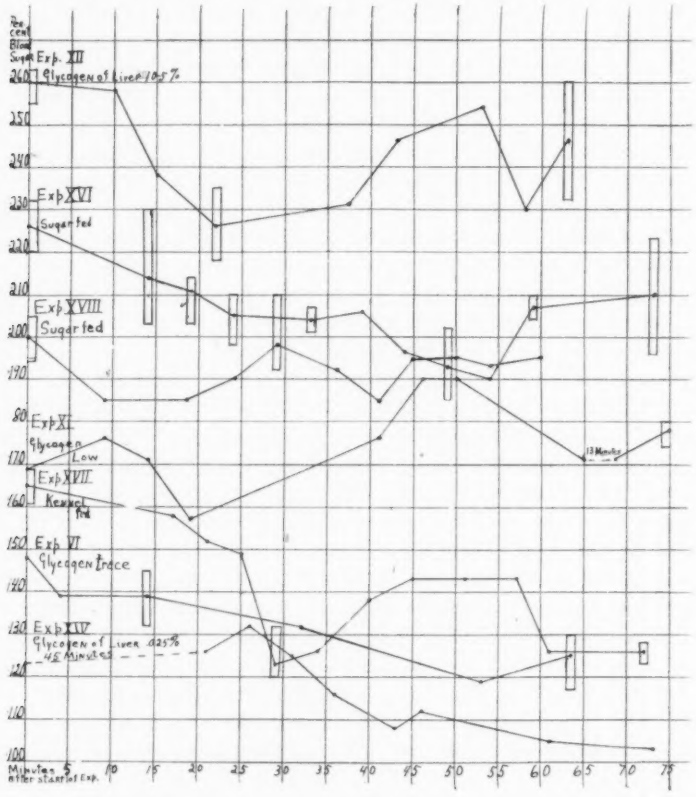

Fig. 1. Curves from seven experiments showing the fluctuations in the sugar level in the blood of the vena cava.

all of these questions can be finally answered, but we have thought it advisable to place what we have on record.

The extent of the fluctuations. For most of the experiments in this connection the modified method of Bang was employed, as described

by one of us (R. G. P.) elsewhere, and the results of seven of them are compiled as curves in figure 1. The extent of the experimental error involved in this method, as indicated here and there along the curves by vertical oblongs, is seen to be considerable. Usually, however, it is below 5 per cent, and frequently duplicate analyses agreed exactly. After allowing for the greatest possible error, it is plain that considerable fluctuations occur, even when the two specimens of blood were removed at intervals of five minutes apart. It will be noticed that the fluctuations are of two types, one, sudden, occurring over a period of a few minutes, and the other, much more gradual.

A. *The sudden fluctuations.* It is probable that these are often due to experimental error, but this is not always the case because they are also present when the much more accurate modified Benedict method is employed. The results of three experiments of the same type in which the estimations were made by this method, are compiled in the curves of figure 2, from which it will be observed that the samples of blood were removed every two or three minutes, instead of every five. This permits us to determine with greater certainty whether these momentary fluctuations actually exist, which, it will be seen, they do, although usually very slight in degree. Thus, in the fifty or so estimations used in the compilation of the curves, the greatest fluctuations occurring in two minutes are a fall from 0.156 to 0.137 per cent (No. 78) and a rise from 0.140 to 0.160 per cent (No. 76). Earlier in this latter experiment two consecutive rises of about 15 per cent each are also present. The former variation occurred immediately after the start of the experiment, and is probably to be attributed to operative

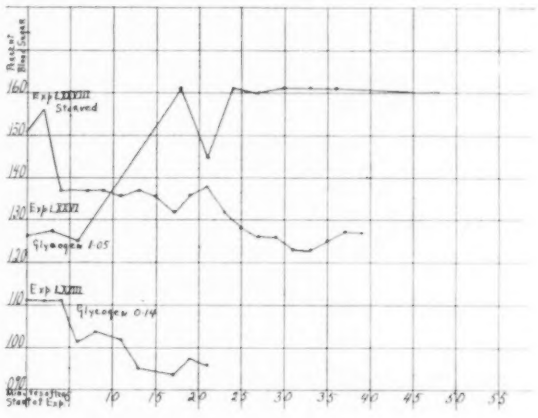

Fig. 2. Curves showing behavior of blood-sugar level compiled from data furnished by a modified Lewis-Benedict method.

disturbances involving the portal circulation. We have observed similar declines in the blood-sugar during the first few minutes of other experiments of this type, so that we always allow a sufficient time for it to occur before attempting to bring about any experimental change. The sudden rises seen in experiment 76 are more serious and we are at a loss to explain them. The animal used in the experiment behaved under anaesthesia in a perfectly normal fashion, and there can be no doubt about the accuracy of the estimations, since blood samples taken from the portal vein gave values which agreed very closely with those taken simultaneously from the vena cava. Unusual though such sudden fluctuations may be, it is essential that their possible occurrence in work of this nature should be allowed for by frequent repetition of the experiments.

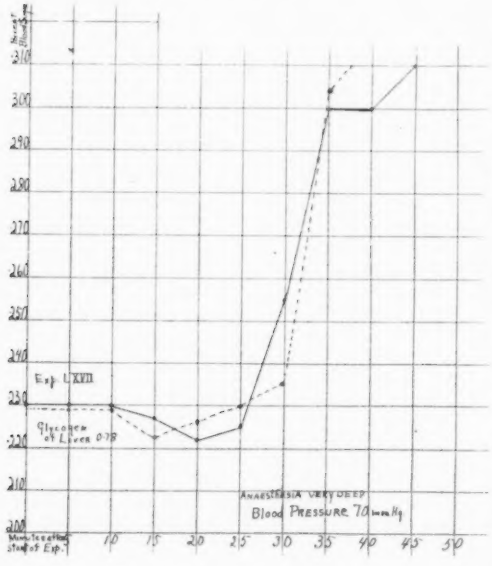

Fig. 3. Curve from experiment in which a sudden fall in blood pressure occurred following a period of faulty anaesthesia.

Such sudden rises in the blood-sugar level occurring unexpectedly during the course of an experiment on an apparently 'normal' animal should be distinguished from similar rises almost invariably to be observed when the animal becomes asphyxiated, or when there is a pronounced fall in arterial blood pressure. In figure 3 curves plotted from the results of such an experiment are given, the animal being hyperglycaemic from the very start.

B. *The gradual changes.* In all the eleven experiments, save the one already referred to (viz., No. 76), there occurred a progressive fall in the blood-sugar level during the first part of the period of observation. This fall lasted for at least thirty minutes, although it persisted for the

whole duration of the observation in about one-half of the experiments (viz., Nos. 6, 14, 16, 73, 78). The gradual decline is probably due to the passing away of conditions which excite the glycogenolytic process during the anaesthetisation and operative manipulation of the animal.

Such evidence that the preparation of the animal causes hyperglycogenolysis, along with the marked variation in the level to start with, might seem to indicate that investigation of the glycogenic function would be impossible in anaesthetized animals, since the already existent disturbance would mask any further change, especially an increase, which might be brought about by experimental interference. It is very probable that conditions which would cause a slight increase of blood-sugar in a 'normal' animal might fail to do so in one that was hyperglycaemic, but this fact does not, in our estimation, justify the conclusion of one author that 'the dog is little suited for investigations concerning glycaemia' (Bang) (6). The emotional glycaemia is at least as marked in the other animals available for such work (cat and rabbit), and, in the case of the rabbit at least, there are much more serious objections to their employment, such as their small size, their herbivorous habits and the impossibility of removal of the pancreas. Unless dogs are employed, investigation of many of the problems concerning the glycogenic function of the liver becomes impossible, but to discount the disturbing influences due to etherization and operative interference we must know exactly the extent and frequency of such disturbance.

The initial level and the subsequent rise in blood-sugar in relationship to the percentage of glycogen in the liver. It is clear from figures 1 and 2 that an initially high level of blood-sugar is more likely to occur in animals having a high percentage of glycogen than in those in which there is only a trace. The secondary rise is also usually more marked in glycogen-rich animals, although it sometimes occurs, as in experiment 11, in those that are glycogen-poor.

The relationship between glycogen-content and the initial sugar-level is well illustrated in the following table (I) taken from other experiments than those used in the compilation of the curves in figure 1.

The relationship between the levels in the blood of the portal vein and vena cava. On account of the magnitude of the blood flow through the liver, the very smallest difference in sugar concentration in the blood entering and leaving it could exist only when an extreme degree of glycogen formation or breakdown existed in the liver. In a subsequent paper we shall submit results showing how such 'percentage' values behave when dextrose is injected into the portal vein. In the present

paper we have collected, in Table II, the results of estimations which were made on samples of blood which were collected from the two vessels either simultaneously or at intervals of not more than one minute apart and prior to the sugar injections.

TABLE I

Relationship between the initial sugar-level and the glycogen-content of the liver.

STARVED			WELL-FED		
Number of experiment	Glycogen in liver	Sugar in blood at start	Number of experiment	Glycogen in liver	Sugar in blood at start
	per cent	*per cent*		*per cent*	*per cent*
31	trace	0.176	32	6.50	0.246
36	trace	0.098	34	0.70	0.163
38	trace	0.090	35	3.62	0.161
41	trace	0.110	37	5.70	0.181
44	trace	0.085	40	1.57	0.200
45	trace	0.075	42	10.37	0.202
49	trace	0.131	47	0.41	0.170
50	trace	0.120	46	9.15	0.118
51	trace	0.118	48	2.00	0.122
65	trace	0.168	53	12.00	0.170
70	trace	0.146	54	4.16	0.205
63	trace	0.130	62		0.150
			68	0.67	0.100
			72	6.5	0.200
	Max mum	0.176		Maximum	0.246
	Minimum	0.075		Minimum	0.100
	Average	0.120		Average	0.170

TABLE II

NUMBER OF EXPERIMENT	SUGAR IN BLOOD OF PANCREATICODUODENAL VEIN	SUGAR IN BLOOD OF VENA CAVA	INTERVAL BETWEEN COLLECTION OF SAMPLES OF BLOOD	
			(1) From same vein	(2) From other vein
	per cent	*per cent*	*min.*	*min.*
65	0.170, 0.168	0.168, 0.167	1	1
70	0.133, 0.133	— , 0.146	1	1
68	0.100, 0.099, 0.100	0.100, 0.098, 0.100	1	1
71	0.137, 0.136, 0.126	0.140, 0.140, 0.140	2	0.5
72	0.200,	0.200, 0.207	2	0.5
74	0.160, 0.148, 0.160	0.160, 0.160, 0.160	2	0.5
75	0.158, 0.138	0.166, 0.158, 0.158	2	0.5

A remarkable correspondence is evident between the two bloods in most cases. When any difference exists it is always small in degree and is, with one exception (No. 70), due to a decline in the portal blood value (Nos. 71, 74, and 75). In the case of No. 74 it is possible that the value 0.148 is due to experimental error, but we have no reason to suspect this in the two other experiments in which the decline occurs (Nos. 71 and 75). In two experiments (Nos. 73 and 76) the estimations were made over longer periods without injecting dextrose into the portal circulation. The results of these experiments are given in the curves in figure 4, from which it will be seen that in one of the experiments (No. 73) there

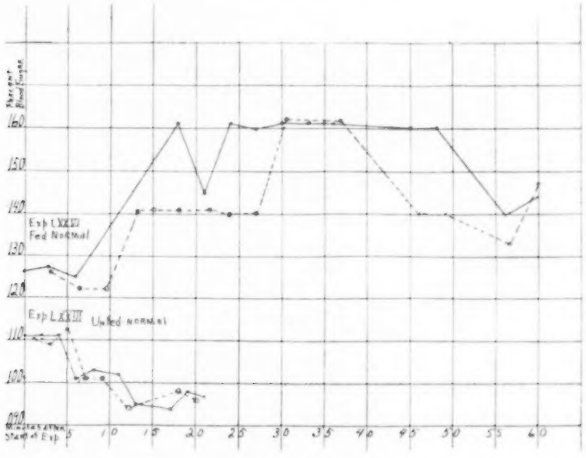

Fig. 4. Curves showing blood-sugar levels in vena cava and portal vein in two dogs which were only anaesthetized.

was entire correspondence, whilst in the other (No. 76) the sugar concentration of the 'cava' blood stood very considerably above that of the portal blood during a period of nearly twenty minutes. This result indicates a high degree of hyperglycogenolysis for which there was no apparent cause. The behaviour of the curve for the portal blood indicates further that the increase of sugar in the blood of the systemic circulation did not in this experiment immediately cause a similar increase in that of the portal vein. The excess of sugar is evidently retained somewhere in the organism, probably in the muscles. In the experiment in which artificial glycaemia was brought about by

injecting dextrose into the portal vein, this delay in the appearance of the excess of dextrose in the portal blood was not noticed (see subsequent communication). We cannot account for it in the present experiment unless we assume that dextrose which has been set free in the liver as a result of hyperglycogenolysis is more liable to be 'fixed' or used in the tissues than injected dextrose. Although there is so far no direct evidence (7) that glycogen-dextrose is dealt with by the tissues in any different manner from chemically pure dextrose, yet it is possible that it is so, and that one of the differences is with regard to its readiness to become deposited in the tissues.

In conclusion we would point out that although the blood-sugar level in an anaesthetised animal does not remain unchanged from time to time, yet the changes occurring during a period of ten minutes are very small when compared with those observed by us on blood similarly removed from animals in which the splanchnic or hepatic nerves were stimulated (8).

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STUDIES IN EXPERIMENTAL GLYCOSURIA

X. The Sugar Retaining Power of the Liver in Relationship to the Amount of Glycogen Already Present in the Organ

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Since the time of Claude Bernard it has been usual to consider the glycogenic function of the liver as one analogous to that of starch formation in plants, that is, as a means for temporarily storing away for future uses in the organism, carbohydrate that is not immediately required. It has been recognized, however, that the capacity of the liver to store glycogen is quite inadequate to account for all the carbohydrate that is assimilated from the intestine, and it is believed that some of the excess is carried to the muscles and deposited there as glycogen, the remainder being either oxidized or more slowly converted into fat. According to the above view, we should expect that the power of the liver to retain sugar as glycogen would be more marked when this organ is empty than when it is already well filled with glycogen. Undoubtedly, such a view is correct when we consider the *total glycogen capacity;* that is to say, the empty liver will hold more than the liver that is already partly filled. But this does not necessarily mean that the *rate* with which the liver converts the sugar to glycogen will be different in the two cases.

Capacity for glycogen and rate of glycogen-formation in the liver need not run parallel. Indeed there are several observations on record which would seem to show that dextrose, absorbed from the intestine, passes more freely into the blood of the systemic circulation in fasting, than in well-fed animals. Thus, Bang (1) and his collaborators have found that after giving from 2 to 10 grams of dextrose by stomach to rabbits, the dextrose concentration of the systemic blood began to rise in fifteen minutes, gaining a maximum in about an hour and then returning to the normal level in three hours. This hyperglycaemia de-

veloped more rapidly and reached a higher level in starved rabbits than in those that had received previous administrations of dextrose; and it usually failed to develop at all in animals that had received and recovered from several previous injections.

Such a result can most easily be interpreted by assuming that the liver more quickly removes the absorbed dextrose when it already contains some glycogen than when it is glycogen-free. If these results can be confirmed, it would indicate that the process which is going on in the liver during starvation is one of glycogenolysis, or more correctly of glyconeogenesis, and that the liver cell cannot immediately reverse it to one of glycogen-formation (glycogenesis) when excess of dextrose appears in the portal blood. The liver cells, in other words, cannot produce glycogen and dextrose at the same time, either when the dextrose comes from their own stores of glycogen or from protein, and it takes some time for the cells to change over from the one function to the other.

The present investigation was undertaken to supply more direct evidence for or against the above hypothesis.

Methods: Dogs were either starved or fed for some days on a diet of bread and meat, or given cane sugar by stomach tube on the evening preceding the experiment. After being anaesthetized with ether, cannulae were placed in the inferior vena cava and, in the later experiments, in the duodenal vein, as described in a previous paper. A cannula filled with Ringer's solution was also placed in one of the branches of the mesenteric vein, as far down as possible. Through the tubing which closed this cannula passed a hypodermic needle connected through a three-way stop-cock with tubes leading to the bottom of two graduated cylinders, one containing Locke's solution, the other, a 4 per cent solution of pure dextrose. The graduates were closed above with stoppers, through a second hole in which they were connected with an 8-litre bottle, in which an air pressure was established by means of a pump. The large capacity of the bottle and the comparatively slow rate of outflow through the hypodermic needle ensured a practically uniform rate of injection during five minutes, and this rate was altered in the different experiments, either by choosing needles of different bore or by changing the air pressure—by means of a mercury valve—in the bottle. Although somewhat hypotonic, the above strength of dextrose was chosen to simplify calculation, since we intended, at the outset, to alter the rate of injection by known degrees during each experiment. The rate of injection varied from 8 to 27.5 cc. in the different experi-

ments, i.e., from 0.32 to 1.2 grams of dextrose in five minutes. Taking the average blood-flow through the portal vein as 1300 cc. in five minutes (calculated on the basis of 4.3 cc. per second for a liver of average weight, 400 grams) (2), and assuming that the blood to start with contained 0.15 per cent dextrose, the above amounts of injected dextrose would give a maximal percentage of 0.25 in the portal blood, supposing none of the dextrose was removed from the blood during the five minutes of injection. This degree of hyperglycaemia in the blood of the portal vein is believed to be not infrequently overstepped during the absorption of carbohydrate, so that the conditions in our experiments in this regard can be considered as well within the normal limits.

In order to ascertain the normal sugar concentration of the blood of each animal, several samples of blood (2 cc. each) were removed at two-minute intervals from the vena cava, and also—in the later experiments —from the duodenal vein. Meanwhile, in most of the experiments, Locke's solution was being injected into the mesenteric vein. The sugar solution was then injected for exactly five minutes, samples of blood being still collected every two or three minutes, and at similar intervals after discontinuing the injection. A second injection period followed after from ten to twenty minutes, unless the condition of the animal was such as to make it inadvisable.

At the end of the experiment a portion of liver was removed and cut in thin slices, which were then dried between filter paper. Duplicate portions, of 10 grams each, were then used to determine the glycogen-content. In some of the earlier experiments a piece of liver was removed, after mass ligation, early in the experiment. It was hoped that results could be secured, from a comparison of the glycogen-content at the beginning and the end of the experiments, that would furnish information as to whether glycogenesis or glycogenolysis was taking place during the time of the observation. So far, the results in this respect have not been satisfactory. Anaesthesia, arterial blood pressure, and the respirations were practically unchanged during the experiments, unless otherwise noted in the tables of results, which follow.

Discussion of results: These are compiled in Tables I and II. It will be most convenient to consider them as a whole first of all, and later to select one or two typical experiments for the purpose of examining more closely the time relationships and the magnitude of the changes produced by the sugar injections.

The figures in the fourth columns of the tables represent the grams of dextrose injected into a mesenteric vein in five minutes; those of the

TABLE I

The effect of dextrose injection into the portal vein of starved dogs on the dextrose-content of the blood of the vena cava

1 NO.	2 WEIGHT	3 FOOD	4 DEXTROSE INJECTED	5 BLOOD-DEXTROSE BEFORE INJECTION	6 BLOOD-DEXTROSE DURING INJECTION	7 AVERAGE INCREASE	8 INCREASE PER 1 GM. DEXTROSE	9 DO. MULTIPLIED BY THE BODY-WEIGHT	10 MAXIMAL INCREASE DURING (D) OR IMMEDIATELY FOLLOWING (A) INJECTION	11 DIFFERENCE BETWEEN AVERAGE PRECEDING AND MAXIMUM DURING	12 GLYCOGEN IN LIVER	13 REMARKS
	kg.		*gm.*	*per cent*	*per cent*	*per cent*	*per cent*				*per cent*	
31	17.3	Starved	1.00	0.176◦	0.255§	0.079	0.079	1.36	0.30D	0.084	Trace	◦Slowly rising. §Did not come back.
36	21.80	Starved	0.88	0.098	0.106§	0.008*	0.009	0.246	0.127D	0.029	Trace	§Hyperglycaemia following.
36a	21.8	Starved	1.00	0.086	0.117◦	0.031	0.031	0.676	0.132D	0.046		◦Came back partly.
38	10.9	Starved	0.72	0.090	0.132	0.042	0.058	0.163	0.158A	0.068	Trace	
38a	10.9	Starved	1.20	0.106	0.112	0.006§	0.005	0.054	0.182D	0.076		§Increased considably after.
41		Starved	0.80	0.110	0.173	0.063*	0.080		0.20D	0.090	Trace	§One estimation.
41a		Starved	0.80	0.104§	0.214	0.110*◦	0.127		0.24D	0.114		◦Came back partially
44	8.17	Starved	0.52	0.085	0.097	0.012	0.023	0.189	0.105D	0.020	Trace	
44a	8.17	Starved	0.40	0.083	0.104	0.021	0.052	0.426	0.115D	0.032	Trace	
45	10.00	Starved	0.24	0.075	0.094	0.019*§	0.079	0.179	0.103A	0.028	Trace	§Did not come back. Dog died.
49	19.00	Starved	0.24	0.131	0.157	0.026	0.108	2.052	0.158D	0.028	Trace	
49a	19.00	Starved	0.44	0.130	0.155	0.025	0.057	1.08	0.162D	0.032	Trace	
50	9.50	Starved	0.64	0.120	0.180	0.060	0.093	0.880	0.195D	0.075		
50a	9.50	Starved	0.48	0.125	0.162	0.037	0.077	0.730	0.169D	0.044	Trace	
51	8.65	Starved	0.32	0.118	0.145◦	0.027*	0.084	0.720	0.164D	0.046		◦Did not come back till after second injection. Died second injection.
65*	5.20	Starved	0.40	0.168	0.214	0.046*	0.115	0.600	0.248D§	0.080		§P. D. & V. C.
70	22.30	Starved	0.48	0.146◦	0.146	None			0.158	0.012		◦One estimation.
71?	6.80	Fed§	0.60	0.140	0.160†	0.020*	0.033	0.22			Trace	§Fed 3 days with special diet; P. D. & V. C. †Partial recovery.
71a	6.80	Fed	0.48	0.184	0.215◦	0.039	0.081	0.55				◦One estimation.

*The asterisks indicate that the percentage of sugar did not return to the normal within five minutes after the injection.

injection.

TABLE II

The effect of dextrose injections into the portal vein of well-fed dogs on the dextrose content of the blood of the vena cava

1	2	3	4	5	6	7	8	9	10	11	12	13
NO.	WEIGHT	FOOD	DEXTROSE INJECTED	BLOOD DEXTROSE BEFORE INJECTION	BLOOD DEXTROSE DURING INJECTION	AVERAGE INCREASE	INCREASE PER 1 GRAM DEXTROSE	DO. MULTIPLIED BY THE BODY-WEIGHT	MAXIMAL INCREASE DURING (D) OR IMMEDIATELY FOLLOWING (A) INJECTION	DIFFERENCE BETWEEN AVERAGE PRECEDING A N D MAXIMUM DURING	PER CENT GLYCOGEN IN LIVER	REMARKS
	kg.		*gram*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*		
32	12.7	Sugar	1.08	0.246	0.265	0.019*	0.017	0.216	0.325A	0.079	6.5	
				0.266	0.284	0.018	0.016	0.203				
34	12.7	Sugar	1.20	0.163©	0.194 $	0.031*	0.026	0.330	0.195D	0.032	0.715	©Results irregular. $ Did not come back.
35	16.3	Sugar	0.80	0.161	0.157	None	None		0.162A	0.00	3.62	
37	13.1	Sugar	0.64	0.181	0.217	0.036 $ *	0.056	0.734	0.294A	0.113	5.7	$ Marked hyperglycaemia steadily developed. *See Curve.*
40	7.27	Sugar	0.64	0.200	0.255	0.055 $ *	0.085	0.618	0.260D	0.060	1.57	$ Only recovered slightly.
40a	7.27	Sugar	0.80	0.232©	0.316	0.084*†	0.105	0.763	0.344A	0.112	1.57	© 1 estimation. †Recovered still less.
42	10.9	Sugar	0.80	0.202	0.245	0.043 $ *	0.050	0.545	0.254D	0.052	10.37	$ No recovery.
42a	10.9	Sugar	0.64	0.253	0.276	0.023 $ *	0.030	0.327	0.276D	0.023		
47	10	Sugar	0.80	0.170	0.170	None $	Decrease		0.170D	0.000	0.412	$ Really hyperglycaemia.
47a	10	Sugar	0.68	0.164	0.141	Decrease	Decrease		0.141D	0.000		
46	10.9	Sugar	0.72	0.118	0.137	0.019	0.026	0.283	0.151D	0.033	9.12	
46a	10.9	Sugar	0.48	0.115	0.153	0.038	0.078	0.850	0.163D	0.048		
48	18	Sugar	0.48	0.122	0.149©	0.027	0.056	1.040	0.260D	0.138	2.00	©Very irregular results. Immediate effect hyperglycaemia.
48a	18	Sugar	0.40	0.140	0.231	0.091†	0.23	4.150	0.280D	0.140		†Start later than usual. Not included.
53	9.9	Bread	0.48	0.170	0.176	0.006 $ *	0.012	0.12	0.188A	0.018	12.00	$ Did not come back.
			0.40	0.235	0.217	Decrease	Decrease					
54	13.5	Bread	0.88	0.205	0.254	0.049*	0.056	0.756	0.262D	0.057	4.16	Altering infusion of Ringer starts here.
			0.96	0.246	0.277	0.031	0.032	0.432	0.316D	0.070		
62	6.5	Bread	0.20	0.15	0.155	0.005*	0.025	0.162	0.165D	0.015		
			0.60	0.169	0.242	0.073 $ *	0.120	0.780	0.27D	0.100		$ Did not come back.
			0.44	0.26	0.260	No change	No change					
68	14.3	Bread	0.64	0.100	0.112 $	0.012	0.019	0.270	0.118D	0.018	0.675	$ Exactly parallel rise in P. V.
72	12.6	Bread	0.48	0.200	0.218 $	0.018	0.0375	0.47	0.220D	0.020	6.5	Hepatic plexus cut. $ Came back increasing later.
72a	12.6	Bread	0.48	0.246	0.309©	0.063	1.31	1.65	0.392A	0.1		©One estimation omitted.
74	8.7	Bread	0.32	0.160	0.176	0.016*	0.031	0.270	0.192D	0.002		
74a	8.7	Bread	0.4	0.176 $	0.191	0.015	0.037	0.322	0.197D	0.021		$ One estimation.

*The asterisks indicate that the percentage of sugar did not return to the normal within five minutes after the injection.

fifth, the percentage of dextrose in the blood of the vena cava immediately preceding the sugar injection. In practically every instance these figures, i.e., in column 5, are the average of determinations on several samples of blood removed at two-minute intervals. The sixth column gives the average percentage of blood-sugar during the injection of the sugar, and the seventh, the average increase. This average increase is nearly always considerably less than the maximal increase, which is given in the tenth columns, because the values in the samples of blood obtained immediately after the injection did not, in most cases, show any increase over the normal. On this account, and also because the first samples taken after discontinuing the injection still showed the increase in sugar, it might have been advisable to use, for the computation of the average, not the values obtained during the injection, but rather those immediately preceding and following the end of the injection period. This method was not chosen because it was found in many cases that the increased discharge of sugar was maintained for some considerable time after discontinuing the injection, thus indicating that a hyperglycogenolysis had become established, probably as a result of the sugar injections. This stimulation of a more or less persistent sugar mobilization will be discussed in a future paper; meanwhile it is important to note that it was more frequent in the case of glycogen-laden livers than in those that were glycogen-free, so that, to have used the figures obtained after the injection, would have made the averages for fed animals too high. The cases in which the sugar percentage did not return to the normal within about five minutes after the injection are indicated by asterisks.

Since figures in the seventh columns represent increases produced by the injection of varying amounts of dextrose into the portal vein, they cannot be compared with one another. To make this possible it was necessary to calculate in each case the average increase for 1 gram of injected dextrose. This has been done by direct proportion—figures in column 8—on the assumption that the same fraction of injected sugar will always be retained by the liver, an assumption for which there is however no experimental justification, for it may well be that, with progressively increasing injections, a greater or a smaller fraction of the sugar is retained. We intend to investigate this point at an early date.

To further reduce the figures to a common basis allowance had of course to be made for the greater quantity of blood in the portal circulation in large, as compared with small animals. Since the total volume

of blood in the body is practically proportional to the body weight, we have made this correction by multiplying the figures in the eighth column by those in the second, since the larger the animal, the greater must be the dilution to which the injected sugar would be subjected. The values thus obtained are more or less arbitrary, but they are comparable one with another. The figures in the twelfth column give the percentage amounts of glycogen found present in portions of liver removed immediately after the animal was killed, or, in some cases, in portions removed before the injection was started.

Although the values obtained after making the corrections as above indicated—i.e., those in the ninth column—do not exhibit as close a correspondence as we had hoped they would, yet, when we compare them in Tables I and II, they seem sufficient to justify the general conclusion that the glycogen-free liver does not remove dextrose from the portal blood any more quickly than one that is glycogen-rich. The ultimate storage capacity of the glycogen-free liver is no doubt greater than that of one that is already partly filled with glycogen, but its avidity for dextrose is certainly not more pronounced; indeed, if anything, it appears to be somewhat less, as a close comparison of the results in the two tables will indicate.

It will be noted that the percentage of dextrose in the blood issuing from the liver at the start of the experiment is very much more constant in the case of the starved animals than in those that were fed. The variation for the former is between 0.075 and 0.146 (leaving out the values in experiments 31 and 65, which are considerably higher) and the average 0.111. The variation for the latter is between 0.100 and 0.206 (leaving out experiment 32) and the average 0.150. This is of course what we should expect, and is entirely in line with our previous experience that operative hyperglycaemia is much more likely to develop in well-fed, as compared with starved animals. The differences, however, render comparison somewhat uncertain, and they make it desirable to compare the extent of the hyperglycaemia produced by 1 gram of dextrose in animals having about the *same* initial blood-sugar percentage. This is done in the following table (Table III).

It will be seen that the dextrose tends to pass the liver more readily in the starved group of animals.

The initial hyperglycaemia probably does not introduce any very serious error, for the increase produced by injecting 1 gram of sugar is at least as marked in initially hyperglycaemic, as in normal animals. Thus, for sugar percentages up to 0.16, the increases were: 0.330, 0.00,

TABLE III

STARVED ANIMALS		WELL-FED ANIMALS	
Original percentage of dextrose in blood	Increase in percentage of dextrose per 1 gram dextrose multiplied by the body weight	Original percentage of dextrose in blood	Increase in percentage of dextrose per 1 gram dextrose injected multiplied by the body-weight
0.098	0.246	0.100	0.270
0.090	0.630		
0.118	0.720	0.118	0.280
0.140	0.220	0.150	0.162
		0.160	0.270
		0.161	0.000
		0.163	0.330
0.168	0.600	0.170	0.000
		0.169	0.120

0.000, 0.283, 0.850, 0.162, 0.27, 0.270; and in those having an initial blood-sugar percentage of 0.200 or above, they were: 0.216, 0.203, 0.618, 0.763, 0.545, 0.327, 0.000.

The uncertain nature of the results of the above experiments led us to suspect that the differences obtained might be due to unequal amounts of sugar entering the liver, for although we always examined to see that the sugar solution was really going into the mesenteric vein, yet it might not become properly mixed with the blood by the time that the hilus of the liver was reached. To control this we performed several experiments in which blood was taken from the portal vein, through a cannula inserted in the central end of the pancreatico-duodenal branch, at about the same time as that collected from the vena cava.

The results of these experiments are given in Table IV, in the sixth, seventh and eighth columns of which the figures standing opposite D represent the percentages of sugar in the blood of the portal vein, and those opposite V, the percentages in that of the vena cava. That is to say, the figures occupying the position of numerators represent the values of the portal blood; the denominators, those of the blood of the vena cava. The numbers are arranged in each column in the order in which the samples of blood were withdrawn from either vein.

It will be seen that, almost without exception, the percentage of sugar in the inflowing and that in the outflowing blood of the liver are equal before any injection of sugar is made (see previous paper). In column 7 the change produced by the injection of sugar is recorded.

TABLE IV

The effect of dextrose injections into the portal vein on the dextrose content of the blood of the portal vein and vena cava

TABLE IV

The effect of dextrose injections into the portal vein on the dextrose content of the blood of the portal vein and vena cava

1	2	3	4	5	6	7	8	9	10
					PER CENT DEXTROSE IN BLOOD SAMPLES				
NO.	WT.	FOOD	GLYCOGEN IN LIVER	DEXTROSE INJECTED IN 5 MIN.	Before injection	During injection	Following injection	INTERVAL BETWEEN SAMPLES FROM SAME VEIN	INTERVAL BETWEEN SAMPLES FROM DIFFERENT VEINS
	kg.		*per cent*	*gr.*					
63	17.4	Starved	Trace	0.84	D 0.13 0.13 0.13 / C *0.13 0.13 0.13	0.143 0.163 0.178 / 0.143 0.163 0.178	0.178 0.143 0.143 / 0.178 0.163 0.162	1 min.	1 min.
65	5.2	Starved	Trace	0.40	D 0.170 0.168 / V 0.168 0.167	0.199 0.248 ..... / 0.167 0.235 0.241	0.212 0.199 0.185 / 0.212 0.226 0.199	1 min. but 2-3 min. for last 3	1 min.
70	22.3	Starved	0.337	0.48	D 0.133 ..... 0.133 / V 0.146	..... 0.141 0.159 / 0.134 0.146 0.158	0.150 0.154 / 0.154 0.166	1 min. but 2-3 min. for last 3	1 min. but 0.5 min. for last 3
70a	22.3	Starved	0.337	0.48	D 0.166 / V 0.166	0.169 0.166 / 0.166	0.182 0.182 0.182 / 0.179 0.179	2-3 min.	0-5 min.
68	14.3	Moderately fed	0.675	0.64	D 0.100 0.099 0.100 / V 0.100 0.098 0.100	..... 0.106 0.111 0.118 / 0.111 0.110 0.111 0.118	0.113 0.108 0.108 / 0.103 0.108 0.107	1 min.	1 min.
68a	14.3	Moderately fed	0.675	0.52	D 0.108 0.111 / V 0.110	..... 0.118 / 0.114 0.118	0.116 / 0.119	1-2 min.	1-2 min.
71	6.8	Well fed?	Trace	0.52	D 0.137 0.136 0.126 / V 0.140 0.140 0.140	0.181 (0.236) / 0.143 0.177	0.148 0.143 0.154 / 0.181 0.170	2 min.	0.5 min.
71a	6.8	Well fed?	Trace	0.48	D 0.194 / V 0.184	0.215 0.215 / 0.143 0.220	0.184 0.140 / 0.196 0.184	2 min.	0.5 min.
72	12.6	Well fed	6.25	0.48	D 0.200 (0.244) / V 0.200 0.207	0.220 0.220 0.220 / 0.217 0.215 0.222	..... 0.220 / 0.200 0.220	2 min.	0.5 min.
72a	12.6	Well fed	6.25	0.52	D 0.220 0.220 / V 0.249 0.243	0.249 0.268 0.333 / 0.200 0.250 0.377	0.350 ..... 0.332 / 0.392 ..... 0.332	2 min. before injection; 1-2 during	0.5 min.
74	8.7	Well fed	4.12	0.52	D 0.160 0.148 0.160 / V 0.160 0.160 0.160	0.190 0.181 / 0.160 0.192	0.167 0.182 0.172 / 0.183 0.186 0.188	2 min.	0.5 min.
74a	8.7	Well fed	4.12	0.40	D 0.176 / V 0.176	..... 0.193 / 0.186 0.197	0.191 0.176 0.148 / 0.197 0.188 0.188	2 min.	0.5 min.
75	5	Well fed	4.88	0.36	D 0.158 0.138 ..... / V 0.166 0.158 0.158	0.207 (stopped breathing; resuscitation) / 0.187		2 min.	0.5 min.
75a	5	Well fed	4.88	0.36	D 0.204 0.204 / V 0.267 0.330	0.345 ..... / 0.345 0.395	0.345 0.332 0.332 § / 0.475 0.489 0.496	2 min.	0.5 min.

*Carotid artery.
§ Blood pressure 40 mm.

In the two-minute interval that was allowed to elapse between the beginning of the sugar injection and the collection of the next sample of blood, a detectable increase in sugar concentration had occurred in the portal blood (i.e., numerators) in nearly every instance. In the case of the cava blood (i.e., denominators), however, the increase in sugar concentration in the two-minute interval was not nearly so frequent (e.g., it was absent in Nos. 65, 70, 70 a, 71, 71 a, 72 a and 74). This delay in the appearance of the injected sugar in the 'cava' blood is very evident in the curves which will be referred to immediately. It seems to occur as frequently in well-fed as in starved animals.

The delay is no doubt partly due to the time occupied by the blood in passing through the liver vessels, but this can not account for all of it, as the following rough calculations will show. In a dog weighing 12 kg., which is about the average of those employed in the experiments, and the liver weight 400 grams, there would be from 1300–1500 cc. of blood passing the organ in five minutes, say 550 cc. in two minutes. Assuming that the liver contains 30 per cent of the total blood in the body (i.e., $\frac{30 \times 800}{100} = 240$ cc.), it will be seen that the blood in the organ before the injection started would have been displaced at least twice during the first two minutes of the injection. Part of the delay must, therefore, depend on diffusion of the excess of dextrose into the lymph and tissue juices of the liver, a diffusion which presumably goes on until blood and tissue juices contain the same concentration of dextrose.

When we compare the sugar concentrations in the samples of blood removed from the two vessels during the last three minutes of the injection periods, they are seen to be practically identical in all of the experiments, except 71 and 72 a. From the neighboring values we are inclined to attribute the result in No. 71 to experimental error. That in No. 72a is undoubtedly due to the progressive hyperglycogenolysis present in this case.

The figures in column 8 furnish us with information regarding the after-effects of the sugar injection, the first samples being usually taken two minutes after discontinuing the injection, and the others at subsequent two-minute intervals. Taking the change in the cava-blood first, it will be seen that in twelve of the experiments (Nos. 63, 65, 70, 70 a, 68, 68 a, 71, 72, 72 a, 74, 74 a, 75) there was no return to the normal level within four or five minutes after discontinuing the injection, such a return being evident only in one experiment (No. 71 a).

Similar comparison of the portal blood reveals a more rapid return

to the normal sugar level in five or six of the experiments (Nos. 63, 65, 70?, 71, 71 a, 74 a), but in the remainder the increase in sugar concentration remained as high as in the cava-blood. These results indicate that there can have been no retention of sugar by the muscles of the body within the time of the observation, so that the blood entering the portal radicles in the intestine contained a concentration of dextrose which was almost equal to that leaving the liver. The dilution due to admixture of this blood with blood from the upper extremities and head was not sufficient to make an appreciable difference. On the other hand, in the experiments in which the sugar level in the portal blood fell more rapidly than that of the cava-blood, it must be concluded that muscle retention of dextrose had occurred. We hope to publish further observations on this question in a subsequent communication. It is of interest to compare this result with that noticed when the increase of sugar in the cava-blood is entirely due to hepatic hyperglycogenolysis (see previous paper).

Returning to our main question, as to whether any difference exists between glycogen-poor and glycogen-rich livers in their avidity for dextrose, we may say that there is no evidence, as judged from the difference between the sugar concentrations of portal and cava-blood, of any difference.

In order to give a clearer presentation of the results than is possible in a table, the curves in figure 1 have been plotted from three of the experiments (LXX, LXXI and LXXIV). The dotted line represents the percentage of sugar in the portal blood, and the discontinuous line, that in the portal vein. The following points should be noted:

1. The quicker rise in sugar concentration in the portal blood than in that of the vena cava.

2. The delay, after discontinuing the injection, in the return to the normal level of the sugar concentration of the blood of the vena cava and frequently also in that of the portal vein.

CONCLUSIONS

The existing glycogen-content of the liver does not demonstrably influence the rate with which this organ removes dextrose from the blood of the portal vein.

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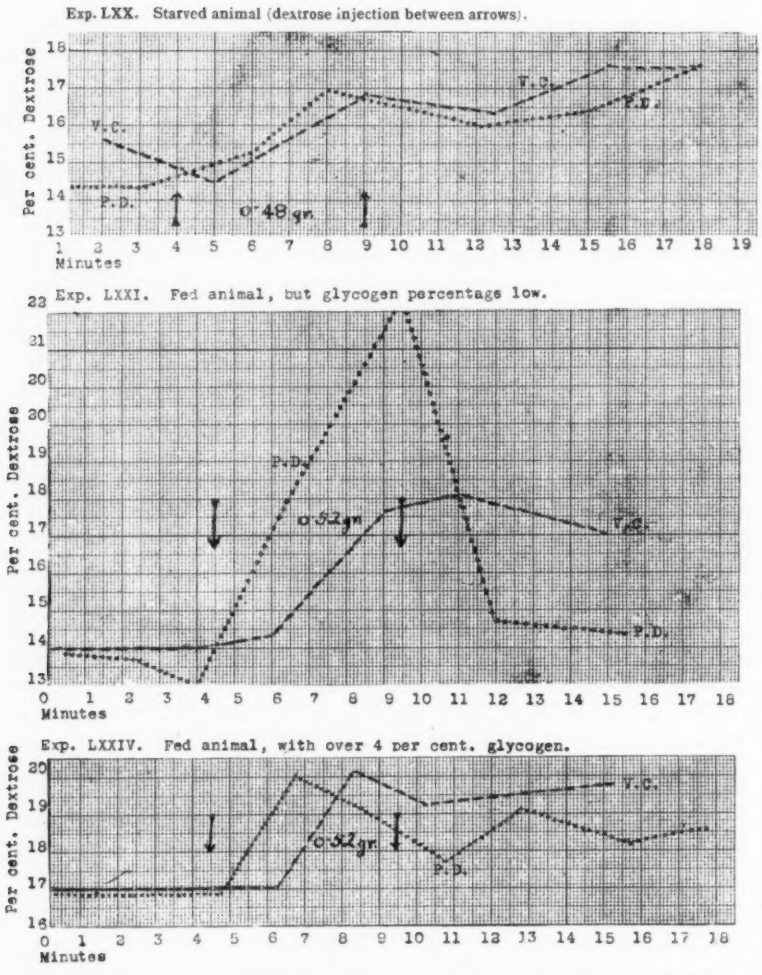

Fig. 1. Curves showing effect of dextrose injections into the portal vein on the sugar content of the blood of the portal vein and vena cava.

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THE DIFFERENTIAL EFFECTS OF ADRENIN ON SPLANCHNIC AND PERIPHERAL ARTERIES

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The opposed action on arterial blood pressure, in the dog and cat, of different amounts of adrenin injected intravenously—a rise, or a rise and fall, after large doses; and a pure fall after small doses—present phenomena of considerable theoretical interest. This is especially true in the relation of adrenin to the sympathetic system, and in view of present rather meager evidence of vasodilator influences effected through that system. In studies previously made in this laboratory[1] it was proved that the same amount of adrenin might have opposed effects under different circumstances. Thus the depressor influence of a given small dose when normal arterial pressure prevails is changed to a pressor influence if the arterial pressure has been carried below a certain level, as, for example, by pithing. These opposed effects indicate that adrenin may operate on antagonistic processes, and, as an alternative to the idea of dilator and constrictor sympathetic actions, the tentative suggestion was offered that adrenin causes relaxation of blood vessels when they are tonically contracted, and contraction when they are relaxed. Another natural opposition in the vascular system which has been claimed by various observers[2] is one existing between the vessels of the splanchnic area and those of the periphery. The present investigation was directed towards an answer to the question whether adrenin has differential effects on these two vascular regions.

The cats used in these experiments were anesthetized with urethane (2.0 g. per kilo, by stomach). The form of adrenin employed was fresh adrenalin diluted with distilled water just before each experiment. Unless otherwise stated the concentration was 1: 100,000. Injections were made into the jugular vein from a syringe graduated to fiftieths of a cubic centimeter. In most cases injections of 0.2 cc. were made, at a

[1] Cannon and Lyman: This Journal, 1913, xxxi, 385.

[2] Dastre and Morat: Système Nerveux Vaso-moteur, Paris, 1884, 329.

uniform rate in each instance, over a period of from fifteen to thirty seconds.

Blood pressure was registered by means of a mercury manometer, connected usually with one of the carotid arteries. In a few cases the pressure changes in the nasal mucosa were recorded by a membrane manometer connected with one of the anterior nares, the other being plugged with vaselined cotton and the posterior nares being closed with Mendenhall's apparatus.[3]

The vasodilator effect of small doses of dilute adrenin is, in our experience, a constant phenomenon rather than "a somewhat variable effect," as Dale[4] describes it. Of the fifty-three animals used in this series of experiments only five failed to show a fall of blood pressure when injected with 0.2 cc. adrenalin, 1:100,000. As will be shown later, four of these were in an abnormal condition which would account for the failure. Of the forty-nine normal cats, therefore, in only one did the small standard dose of adrenalin fail to cause a drop of arterial pressure.

The response of peripheral arteries. In order to determine the response of the peripheral arteries to this small dose of adrenalin, the circulation was excluded from the splanchnic region by tying the inferior and superior mesenteric arteries and the coeliac axis, and sometimes also the renal arteries. Of twenty-three animals with splanchnic vessels thus tied, fourteen still showed the characteristic fall of arterial pressure after injection of the standard dose. In four animals in which there had been a preliminary rise of pressure followed by a fall the same dose failed to cause the rise after the splanchnic vessels had been excluded. In two, showing only a rise previous to the tying, the adrenalin produced only a fall after the tying. And in another case a fall of 20 mm. (14 per cent) before splanchnic exclusion was increased to 38 mm. (32 per cent[5]) afterwards.

These results clearly indicate that a small dose of adrenalin (0.2 cc., 1:100,000) causes relaxation of the peripheral arteries. In no case did constriction result. That in some instances the fall was not increased after splanchnic exclusion was probably due to lessened tone in the peripheral arteries as a result of the operation. With such lessened tone the adrenalin would probably not have so great an influence as in conditions of greater tone. This explanation is supported by the obser-

[3] Mendenhall: This Journal, 1914, xxxvi, 59.

[4] Dale: Journ. Physiol., 1913, xlvi, 291.

[5] The blood pressure which had been 143 mm. before tying the splanchnic vessels was lowered to 118 mm. by the operation.

vation that immediately after the operation the standard dose of adrenalin evoked no response, but as time elapsed it brought forth the characteristic dilation.

The response of splanchnic arteries. To find the response of splanchnic arteries the abdominal aorta above the iliacs, both subclavians and both carotids were tied. There remained for the circulation the vessels supplying the trunk and the thoracic viscera as well as those of the splanch-

TABLE I

Effect of excluding the limb and neck arteries upon the response to 0.2 cc. adrenalin, 1:100,000. Pressures are in mm. of mercury. The same dose of adrenalin was given in each case.

BEFORE TYING				AFTER TYING PERIPHERAL ARTERIES		
Cat	Pressure	Adrenalin caused a fall of	Per cent fall	Pressure	Adrenalin caused a rise of	Per cent rise
	mm.	*mm.*		*mm.*	*mm.*	
I	104	23	22.1	150	2	1.3
				72	7	9.7
II	154	21	13.6	157	11(fall)	7.0 (fall)
				104	6(rise)	5.7 (rise)
III	135	9	6.7	131	10	7.6
IV	115	30	26.0	81	12	14.8
V	113	17	15.0	99	11	11.1
VI	110	20	18.1	55	14	25.4
VII	124	28	22.5	75	30	40.0
VIII	112	32	28.5	131	8	6.1
				80	26	23.5
IX	109	12	11.0	140	12	8.5
X	108	16	14.8	44	17	38.6
XI	112	24	21.4	60	8	13.3
XII	106	20	18.8	108	15	13.8
XIII	94	32	34.0	107	23	21.4
XIV	132	36	27.2	95	4	4.2
XV	138	18	13.0	140	16	11.4
XVI	90	26	28.8	48	7	14.5

nic region. Adrenin is without effect on the pulmonary arteries;[6] it typically causes relaxation of the coronaries,[7] but since the coronary vessels are not capacious they need not be considered. So far as the blood supply to the trunk is concerned, it is probably of much smaller

[6] Brodie and Dixon: Journ. Physiol., 1904, xxx, 488.

[7] Langendorff: Zentralbl. f. Physiol., 1907, xxi, 555; Cow: Journ. Physiol., 1911, xlii, 132.

volume than that of the large and extensive abdominal organs.[8] It is justifiable, therefore, to regard the animal operated upon as described above as carrying on mainly a splanchnic circulation, and to consider the influence of adrenin as exerted chiefly upon the splanchnic vessels. The limb and neck arteries were tied in twenty animals. The operation invariably resulted in a marked increase in arterial pressure.

In all but two of the twenty cases the standard dose of adrenalin (0.2 cc., 1: 100,000), injected after the limb and neck arteries were tied,

TABLE II

Effect of excluding alternately the peripheral and splanchnic arteries upon the response to 0.2 cc. adrenalin, 1:100,000. Blood pressure is expressed in millimeters of mercury. The Roman numerals show the order of clamping and unclamping the arteries.

	NORMAL			PERIPHERAL ARTERIES CLAMPED			UNCLAMPED			SPLANCHNIC ARTERIES CLAMPED		
Cat.	Pressure	Adrenalin caused fall of	Per cent fall	Pressure	Adrenalin caused rise of	Per cent rise	Pressure	Adrenalin caused	Per cent	Pressure	Adrenalin caused fall of	Per cent fall
	mm.	*mm.*		*mm.*	*mm.*		*mm.*	*mm.*		*mm.*	*mm.*	
				I			II			III		
A	124	27	21.7	73	32	43.8	83	19 fall	22.8 fall	110	10	9.1
				III			II			I		
B	111	17	15.3	73	5	6.8	68	5 rise	7.3 rise	No effect		
				III			II			I		
C	112	32	28.5	132	8	6.0	118	17 fall	14.4 fall	128	15	11.8
				80	26	32.5						
				I			II			III		
D	135	18	13.3	132	9	6.8				113	36	31.8

caused a rise of arterial pressure—an effect just the opposite of that caused by the same dose before the tying. Often when the pressure had been greatly increased by the tying, the standard dose at first produced a fall of pressure, but later, as the pressure lessened, the fall was changed to a rise. Moreover, as the pressure decreased below normal the percentage rise after the standard dose usually increased.

In Table I the figures show the change from a fall to a rise when the peripheral arteries are tied.

[8] Ranke: Die Blutvertheilung, Leipzig, 1871, 69.

Splanchnic and peripheral effects in the same animal. In four animals both splanchnic and peripheral responses were obtained after adrenalin injection by clamping first one group of arteries and then the other after the first had been released. The splanchnic arteries were clamped first in two cases, and the peripheral arteries were clamped first in the other two. The results are shown in Table II. In each case the dose of adrenalin was 0.2 cc., 1:100,000.

In every case clamping of the peripheral arteries, whether primary or secondary to splanchnic exclusion, caused the vascular response to the standard dose of adrenalin to change from the normal relaxation (fall) to contraction (rise) (see fig. 1). And in every case except one (B),

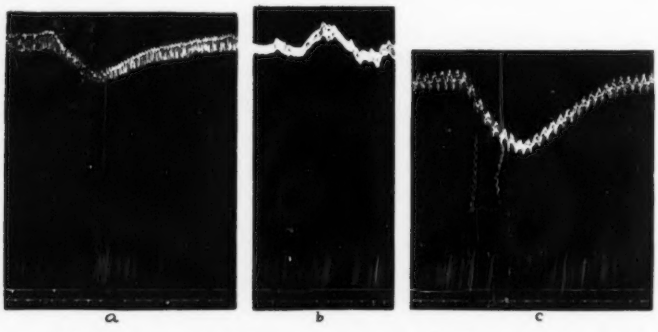

Fig. 1. Effect of injecting 0.2 cc. adrenalin, 1:100,000: a. Upon the whole circulation; b. Upon the arteries of the splanchnic area (peripherals clamped); c. Upon the arteries of the limbs (splanchnic arteries clamped). Time interval five seconds. Middle line, pressure base line (0 mm).

either primary or secondary clamping of the splanchnic arteries continued the normal drop in pressure resulting from the standard dose of adrenalin, though the drop in some instances was not so great as before. In D, however, the fall of 13 per cent under normal conditions was increased to 31 per cent after the splanchnics were clamped. The failure of effect in B may have been due to a too-great relaxation of the vessels as a result of the operation In A and C after the release of the peripheral and the splanchnic vessels adrenalin produced the usual fall. The rise in B may be explained as a splanchnic effect, in the presence of relaxed peripheral vessels (note the low pressure, 68 mm.).

Cases in which dilute adrenalin caused a rise in blood pressure. Occasionally there are animals in which no fall in arterial pressure results

from doses of adrenin which normally cause a fall, nor can a fall be obtained by diminishing the dose. As already stated, five cases out of fifty-three were found in which no fall occurred after the standard dose. It will be recalled that all but one of these animals were in poor physical condition. One animal had been suffering from urethritis for about three weeks and had eaten insufficiently during that time. It had decreased in weight from 2.9 to 2.4 kilos. The blood pressure was 58 mm. of mercury; 0.2 cc. adrenalin, 1: 100,000, injected at the same rate as in other experiments resulted in 17–18 mm. rise in blood pressure. Even 0.2 cc. adrenalin 1: 1,500,000, caused in some tests a slight rise. In no test was the slightest fall in pressure obtained.

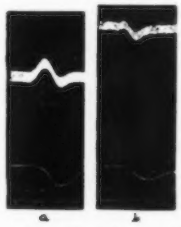

Fig. 2. Effect of 0.2 cc. adrenalin, 1:100,000 upon a cat that was ill: a. Upon whole circulation; b. Upon peripheral arteries (splanchnics clamped). Nasal plethysmograph line just above pressure base line (0 mm). Time line at bottom, interval half minutes. A fall in plethysmograph curve indicates constriction.

In a second case the animal was pregnant and appeared poorly nourished, for its ribs were very prominent. The blood pressure was about 95 mm. of mercury, and nothing but a rise could be induced, even with as little as 0.02 cc. adrenalin, 1:100,000. The arteries of the splanchnic area were tied, whereupon five or six times as much adrenalin was required to cause a rise equivalent to that obtained before the occlusion of the splanchnic arteries (sufficient time was allowed to elapse for the recovery of vasomotor tone). Apparently the primary rise had been due largely to contraction of the splanchnic area and the remainder of the arterial system was not responsive, in the direction of contraction, to the standard minute dose.

A third animal, whose blood pressure was but 67 mm. of mercury, was diseased; in this animal 0.2 cc. adrenalin, 1: 100,000, caused a rise of 7 mm. (fig. 2), but after the splanchnic arteries were tied the same dose caused a fall of only 3 to 6 mm. This experiment, therefore, indicates that the rise, with very dilute adrenin, in animals in weakened condition, is due to constriction of the splanchnic arteries and also to lessened dilation of the peripheral vessels.

A fourth animal which had been given alcohol, failed to show a definite fall with dilute adrenalin.

No explanation was to be had for the failure of the depressor effect in the fifth animal.

In three of the five, however, there was clear evidence of a low blood pressure, a condition that harmonized with the atonic state of the animals. The indication that toneless peripheral vessels are not made to relax further by injections of adrenin has already been mentioned. It seems reasonable to assume, therefore, that when animals are in poor physical condition, a small dose of adrenin fails to cause relaxation of the vessels that normally relax for such a dose, because the tone of these vessels is already low.

Depressor action of adrenin as affected by low arterial pressure. If low arterial pressure was the occasion for failure of adrenin to produce a fall of arterial pressure in the foregoing cases, it becomes a matter of interest to learn whether an induced low pressure will affect the action

TABLE III

Effect of lowering the arterial pressure, by hemorrhage, and then raising it, by restoring the lost blood, upon the response to the standard dose of adrenalin

BLOOD PRESSURE IN MM. OF MERCURY	CHANGE IN PRESSURE IN RESPONSE TO 0.2 CC. ADRENALIN 1:100,000	PER CENT CHANGE
mm.	*mm.*	
102	14 fall	13.7 fall
83 from hemorrhage	6 fall	7.2 fall
71 from hemorrhage	5 rise	7.0 rise
65 from hemorrhage	8 rise	12.3 rise
77 from injection	4 rise	5.1 rise
87 from injection	4 rise	4.6 rise
94 from injection	4 rise 2 fall	2.1 fall
99 from injection	5 rise 5 fall	5.0 fall
109 from injection	2 rise 12 fall	11.0 fall

of the substance. Low pressure was established in two different ways, by hemorrhage, and by depressor stimulation.

(*a*) *Low pressure due to hemorrhage.* In seven experiments there was one in which the primary fall of arterial pressure due to injected adrenalin was changed to a rise as the pressure was reduced by hemorrhage from 102 mm. to 65 mm. The drawn blood was defibrinated and returned to the circulation by degrees. As the pressure rose in consequence of the restored blood the depressor action of adrenalin gradually reappeared.

Table III shows in this case the change in response to 0.2 cc. adrenalin, 1:100,000, as the pressure was lowered and then raised again by injection of defibrinated blood.

In the remaining six experiments there was merely a smaller fall, following the standard dose of adrenalin, as the blood pressure was lowered by bleeding, until at about 40–50 mm. there was either no effect or a slight rise and fall. That no considerable rise occurred may have been due to a temporary abolition of the splanchnic response as a result of hemorrhage, for very little time elapsed between the bleeding and the injection.

The effect of the standard dose of adrenalin was next studied in animals with splanchnic circulation excluded and with blood pressure lowered by hemorrhage. In the five cases studied nothing but a fall in pressure occurred even when the pressure had been lowered to 40 or 50 mm. (fig. 3). At 20 to 30 mm. the dose had no effect. The figures in

TABLE IV

Response to the standard dose of adrenalin in an animal with the splanchnic arteries tied, when the pressure is lowered by hemorrhage

BLOOD PRESSURE IN MM. OF MERCURY	RESPONSE TO 0.2 CC. ADRENALIN 1:100,000	PER CENT FALL
mm.	*mm.*	
161	12 fall	7.4
135	12 fall	8.8
129	18 fall	13.9
110	13 fall	11.8
97	15 fall	15.4
84	13 fall	15.4
71	18 fall	25.3
50	10 fall	20.0
34	6 fall	17.6

Table IV present a good example of the response to adrenalin (0.2 cc., 1:100,000) when in an animal with splanchnic circulation excluded, the blood pressure is decreased by hemorrhage.

Judging from the experiments described earlier in this paper one would expect, in an animal with the peripheral arteries tied, that the rise in pressure, in response to adrenin would continue as the pressure was lowered by bleeding. This was found to be the case in the only animal in which it was tried. The rise with 0.2 cc. adrenalin persisted with the pressure as low as 10 mm. (fig. 4). At 70 mm. pressure the dose caused 8 mm. or 11.4 per cent rise. At 29 mm. the same dose caused 8 mm. or 27.5 per cent rise. At 10 mm. the same dose caused 3 mm. or 30 per cent rise.

Fig. 3.

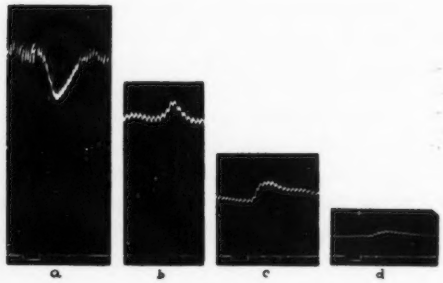

Fig. 4.

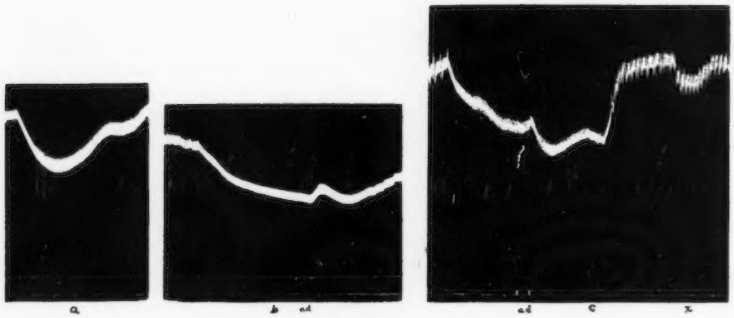

Fig. 5.

Fig. 3. Effect of 0.2 cc. adrenalin, 1: 100,000 after hemorrhage, with arteries of splanchnic area tied: a. Before bleeding; b. 65 cc. of blood removed; c. 35 cc. additional blood removed. Time half minutes. Lower line pressure base line (0 mm).

Fig. 4. Effect of 0.2 cc. adrenalin, 1: 100,000 after hemorrhage with peripheral arteries tied: a. Before tying peripheral arteries; b. After tying peripheral arteries; c. 35 cc. of blood removed; d. 28 cc. additional blood removed. Time interval half minutes. Base line zero pressure.

Fig. 5. Effect of 0.2 cc. adrenalin, 1: 100,000 when blood pressure is lowered by stimulation of the depressor nerve: a. Without depressor stimulation; b. Pressure lowered by depressor stimulation, showing rise with adrenalin; c. Pressure lowered by depressor stimulation, showing fall with adrenalin (different animal). Adrenalin injected at *ad*. *x* shows effect of same dose of adrenalin with normal pressure. Time half minutes.

(*b*) *Low pressure due to depressor stimulation.* When blood pressure is lowered by stimulation of the depressor nerve, 0.2 cc., adrenalin, 1: 100,000, may cause, as shown by Cannon and Lyman,[9] a fall, a rise and fall or a pure rise depending to a certain extent upon the height of the pressure when injected (fig 5). In cases where a rise occurs, it disappears if the splanchnics are tied. Seven animals were used in

TABLE V

Effect of stimulation of the depressor nerve upon the response to the standard dose of adrenalin (0.2 cc., 1:100,000). Pressures are expressed in mm. of mercury.

NORMAL				AFTER DEPRESSOR STIMULATION				
							Splanchnics tied	
Animal	Pressure	Effect of adrenalin	Per cent change	Pressure from depressor stimulation	Effect of adrenalin	Per cent change	Pressure from depressor stimulation	Effect of adrenalin
	mm.	*mm.*		*mm.*	*mm.*		*mm.*	
A	108	3 rise 13 fall	12.0	65	9 rise	13.8	74	No effect
B	115	30 fall	26.0	85	14 rise	16.4	114 (158 mm. after tying splanchnics)	14 fall
C	122	10 fall	8.1	67	19 rise	28.3	80	No effect
D	120	19 fall	15.8	93	15 rise 7 fall	5.3 7.5		
E	89	28 fall	31.4	43	5 rise	11.6		
F	126	12 fall	9.5	90	13 fall	14.4		
G	102	15 fall	14.7	90 73 49	[2 rise [13 fall [4 rise [4 fall 3 rise	[2.2 [14.4 [5.4 [5.4 6.1	42	No effect

studying the effect of dilute adrenalin when pressure was lowered by depressor stimulation. In every case both vagi were cut. Standard doses of adrenalin (0.2 cc., 1: 100,000) were invariably injected. Table V shows the results of these experiments.

As the results in Table V indicate, exclusion of the splanchnic area causes adrenin to have no pressor effect when the general blood pressure

[9] Cannon and Lyman: Loc cit., 387.

has been lowered by depressor stimulation to a point where the standard dose would naturally produce a rise.

In the foregoing experiments the evidence obtained by simple tying out of the splanchnic region or of the neck and limb vessels has been supported. If arterial pressure has been lowered either by hemorrhage or by depressor stimulation the standard dose of adrenin, instead of producing a fall of pressure, produces a rise or has no effect. If now the splanchnic area is excluded adrenin fails to have any pressor influence and may indeed drop the pressure still further. If, on the contrary, the peripheral arteries are tied, the same dose causes an elevation of the lowered pressure, even when 10 mm. measures the arterial tension.

Latent period and duration of the adrenin effects. Since the splanchnic and peripheral arteries respond in opposite directions to minute doses of adrenin, and yet the end result, when both portions of the circulation are affected, is the characteristic fall of pressure which is seen when the peripheral vessels alone are involved, the time relations of the response in the two vascular regions becomes a question of considerable interest. Tests were made on eight animals, and by records written on a rapidly moving drum the latent period and the duration of the adrenin effect were carefully estimated. The results are presented in Table VI.

In the cases of a pure drop in pressure the latent period varied from 12.5 to 22 seconds, with an average of 15.7 seconds. The average latent period for the splanchnic rise (after tying the peripheral arteries) was 18.6 seconds. It seemed probable, therefore that with a minute dose of adrenin, peripheral dilation precedes splanchnic constriction and consequently masks it. There is a possibility, however, that the operation of tying or clamping off part of the circulatory system tends to lengthen the latent period—a suggestion that receives support from an observation that a fall of pressure which occurred in 12.6 seconds before operation did not occur after splanchnic exclusion until 26 seconds had elapsed.

If the foregoing suggestion is correct, the discrepancy between the latent periods of the peripheral relaxation and the splanchnic contraction would not be so great as the figures in Table VI indicate. Indeed in two of the cases there was first a rise of blood pressure and then a fall. With somewhat larger doses of adrenin than those here given this is a usual result. Since the standard dose causes both a splanchnic contraction and a peripheral relaxation of arteries, the common occurrence of a pure fall of pressure, when both these parts of the circulatory system are affected, indicates that this small amount of adrenin usually affects first the peripheral vessels.

The duration of the fall in pressure after the standard dose (0.2 cc. adrenalin, 1: 100,000) averaged in five animals 59 seconds. The average duration of the splanchnic rise in five animals, however, was only 37.8 seconds. In no single case did the splanchnic rise last more than three-fourths as long as the normal fall. With the amount of adrenin used in these experiments, therefore, the dilation of the peripheral arteries begins earlier and lasts longer than the constriction of the splanchnic arteries. The result is that the splanchnic rise is masked by the peripheral fall of pressure.

TABLE VI

Latent period and duration of adrenin effects. In each case 0.2 cc. adrenalin, 1:100,000, was injected. Records were made on a rapidly moving drum

ANIMAL	WEIGHT IN KILOGRAMS	LATENT PERIOD OF THE NORMAL FALL	DURATION OF FALL	LATENT PERIOD OF THE SPLANCHNIC RISE (PERIPHERALS TIED)	DURATION OF RISE
		sec.	*sec.*	*sec.*	*sec.*
A	2.5	13	70	17	42
B	2.0	12.6	53	17.5	38
C	3.2	15	46	18	31
D	2.5	12.5	66	23	41
E	2.1	(rise 8)			
		fall 15	(52)	18	37
F	2.7	20			
G	3.0	(rise 14)			
		fall 22	(48)		
H	3.2	16	59		
Average		15.7	59	18.6	37.8

Effect of dilute adrenin on the blood vessels in the nasal mucosa. In view of the opposite effects of dilute adrenin upon the splanchnic and peripheral arteries, the question arose as to whether the dilation of the peripheral vessels extended to the skin or was limited to the more deeply lying vessels. No convenient method was found to determine the action of vessels in the skin areas over the general body surface, but the vascular changes in the nasal mucosa were easily studied. A membrane manometer was connected to one of the nasal openings in the manner described earlier in this paper. The nasal plethysmograph record was made simultaneously with the tracing of general blood pressure.

Doses of adrenalin (0.2 cc., 1: 100,000) which caused dilation of the peripheral arteries always caused constriction of the nasal mucosa (fig. 2). Amounts as small as 0.05 cc. of adrenalin, 1: 100,000, caused constriction. Martin and Mendenhall[10] produced constriction of the nasal mucosa by injecting 0.5 cc. adrenalin, 1: 1,000,000. The reaction of the nasal mucosa to adrenin therefore is of the splanchnic type rather than the peripheral type.

The threshold dose of adrenin changing a fall of blood pressure to a rise. In determining the threshold dose it is necessary to consider the rate[11] of injection as well as the concentration. And since the sensitiveness of tissues to adrenin decreases if the injections are too frequent or too concentrated,[12] care must be exercised to avoid these sources of error.

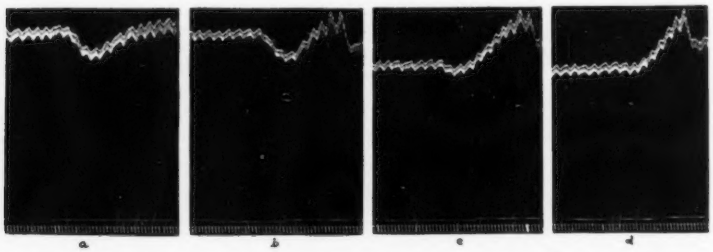

Fig. 6. Threshold of the change from a fall to a rise with adrenalin when splanchnic arteries are tied: a. 0.1 cc. adrenalin, 1: 10,000, fall only; b. 0.25 cc. adrenalin, 1: 10,000, fall and rise; c. 0.3 cc. adrenalin, 1: 10,000, slight fall, large rise; d. 0.35 cc. adrenalin, 1: 10,000, rise only. Time seconds.

The rate of injection was kept nearly constant, being uniform and over a period of from ten to twenty seconds, For convenience two concentrations of adrenalin solution were kept ready, viz., 1: 100,000 and 1: 10,000. To prevent dilution in the process of changing the solutions one solution was injected into the external jugular vein while the other was injected into the femoral vein. In order to avoid the lowering of the sensitiveness of the blood vessels to adrenalin the smallest possible number of doses were injected.

As the evidence already presented has shown that adrenin causes constriction of the arteries of the splanchnic area and relaxation of those of the periphery, the question of the threshold dose of adrenin, changing

[10] Unpublished work done in this laboratory.

[11] Cannon and Lyman: Loc cit., 382.

[12] Elliott: Journ. Physiol., 1905, xxxii, 443.

a fall to a rise, is concerned solely with the effect on the peripheral vessels.

The threshold was determined for three animals, the splanchnic arteries being tied off in each case. The threshold for two of these was between 0.2 cc. and 0.3 cc. of 1: 10,000 adrenalin while in the third it was about 0.1 cc. of 1: 10,000 adrenalin. The third animal weighed but 2.0 kilos while the others weighed 3.7 and 3.4 kilos. As the threshold was just passed a preliminary fall preceded the rise (fig. 6). The threshold of the change of a fall to a rise apparently varies with the individual. The experiments were too few in number to permit definite conclusions but they indicate the magnitude of the threshold.

Fig. 7. Effect of small doses of ergotoxine phosphate in increasing response of splanchnic arteries to adrenalin (peripheral arteries tied): a. Effect of 0.2 cc. adrenalin, 1: 100,000, before giving ergotoxine; b. Effect of same dose of adrenalin after giving two doses of ergotoxine 0.5 mgm. and 0.6 mgm., respectively. Time half minutes.

Reversal of the adrenin effect after ergotoxine. Dale's[13] discovery, that after ergotoxine a dose of adrenin that would normally cause an increase of arterial pressure causes a decrease, is of considerable interest in connection with the reversed effects of adrenin on peripheral and splanchnic arteries. Since the alteration induced by ergotoxine is from contraction to relaxation it was necessary to make the test on animals with the peripheral arteries tied. Under these circumstances does ergotoxine cause the usual increase of pressure resulting from the standard dose of adrenin to change to a decrease?

It was impossible to obtain the "reversal effects" after the injection of small doses of ergotoxine phosphate (e.g., 0.5 mgm.). Indeed these small doses seemed to render the splanchnic response more sensitive, for the rise of pressure after adrenalin injection was thus increased (see fig. 7). Reversal or inhibition of the splanchnic constriction was obtained only after several larger doses (5 to 10 mgm.) of ergotoxine, such as Dale used, had been administered (see fig. 8). Thus after such large doses, adrenalin (0.4 cc., 1: 10,000) caused, in an animal with limb and neck arteries tied, a fall of arterial pressure from 86 mm. to 65 mm. Recovery required six minutes. When 1 cc. of this solution was injected there was a similar fall, which was recovered from after eleven minutes.

[13] Dale: Journ. Physiol., 1905, xxxii, lix.

Discussion of the opposed action of splanchnic and peripheral arteries in response to dilute adrenin. Cannon and Lyman[14] proposed the idea that adrenin causes relaxation of the blood vessels when they are tonically shortened,—contraction when they are relaxed. The evidence in this research indicates that the opposite action of dilute adrenin depends rather upon opposite effects produced in the splanchnic and periperal arteries. The rise of pressure when the vessels are relaxed would thus be explicable on the ground that the standard dose of adrenin cannot relax further the peripheral vessels already relaxed but can still have its constrictor influence on the splanchnic area. In certain conditions, however, the state of tonicity may play a part, e.g., when the blood

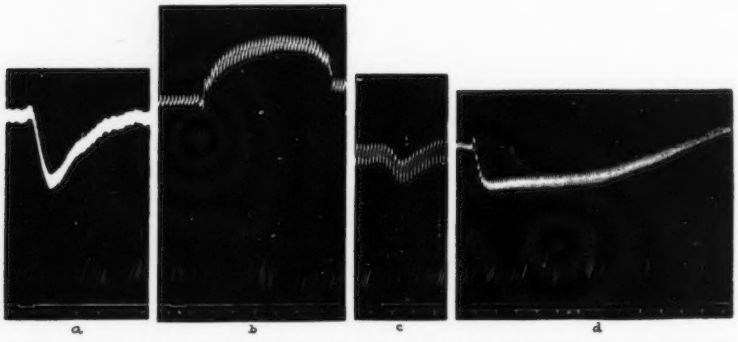

Fig. 8. Reversal of splanchnic arterial response to adrenalin after giving large doses of ergotoxine phosphate: a. Effect of 0.2 cc. adrenalin 1:100,000 before tying arteries; b. Effect of 0.2 cc. adrenalin 1:100,000 after tying peripheral arteries; c. Effect of 0.2 cc. adrenalin 1:100,000 after 21.5 mgm. ergotoxine had been given. d. Effect of 0.4 cc. adrenalin, 1:1,000 after a total of 27.5 mgm. ergotoxine had been given.

pressure has been rendered unusually high by clamping the peripheral arteries, for dilute adrenin then often causes dilation. But even in this case, the dilation might be due to the arteries supplying the trunk, as it is impossible to tie them off.

Although there is no convincing proof that there are vasodilator nerves in the sympathetic system, their presence in the peripheral blood vessels and their absence from, or relatively slight development in, those of the splanchnic area, would offer the most plausible explanation of the differential effects of dilute adrenin. An alternative explanation would

[14] Cannon and Lyman: Loc cit., 398.

be that the arterial muscle differed in the two regions and that adrenin produced its effect by direct stimulation of the plain muscle. The first seems impossible, while the second is contradicted by evidence of Dixon and Brodie[15] that adrenin produces its effect through the sympathetic nerve ending.

If the "sympathetic vasodilator" hypothesis is accepted it is necessary to assume that the vasodilators are more sensitive to adrenin than are the vasoconstrictors, in order to account for the change from a fall to a rise in the response of the peripheral arteries to increasing doses of the drug. The rise which occurs as the vasoconstrictor threshold is passed is preceded by a fall (see fig. 6). This would be expected from adrenin slowly injected because the first amounts reaching the nerve endings might be insufficient to stimulate the less sensitive constrictor endings yet strong enough to affect the vasodilator endings and then as the amount of adrenin increased the vasoconstrictors would be brought into action and overwhelm the vasodilator effect.

The idea that vasodilator and vasoconstrictor nerves can be brought into action in turn by different strengths of stimulation receives support from the observation that weak sensory stimulation may cause a lowering[16] of blood pressure while strong sensory stimulation (20 to 200 times stronger than the stimulation which produces a depressor effect) usually produces a rise.[17] Bowditch and Warren[18] found that a slow rate of stimulation with an induced current caused vasodilation while a more rapid rate caused vasoconstriction. Vasodilators may have been stimulated in the former case and vasoconstrictors in the latter case.

Ostroumoff[19] was one of the earliest to believe that the sympathetic contained vasodilator nerves. He was supported in this belief by Puelma and Luchsinger.[20] Dastre and Morat[21] thought that they had evidence of the existence of vasodilators in the sympathetic. Dale[22] is inclined to accept the theory of the existence of an admixture of vasodilators and vasoconstrictors in the sympathetic and, as he points out,

[15] Dixon and Brodie: Loc cit., 494.

[16] Knoll: Sitzungs. a. Akad. d. Wissensch. zu Wien, Math.-Naturwiss. Klasse, 1885, xcii, Abtheilung, 3, 449.

[17] Martin and Lacey: This Journal, 1914, xxxiii, 222.

[18] Bowditch and Warren: Journ. Physiol., 1886, vii, 447.

[19] Ostroumoff: Pflüger's Arch., 1876, xii, 219.

[20] Puelma and Luchsinger: Pflüger's Arch., 1878, xviii, 489.

[21] Dastre and Morat: Loc cit., 247.

[22] Dale: Loc cit., 299.

this would account for the fact that no reversal can be obtained in the rabbit, vasodilators in the sympathetic being theoretically absent.

The utility of the simultaneous peripheral dilation and splanchnic constriction. It may be that the amount of adrenin, which is poured into the blood stream during times of stress or excitement, is at first of the order of that used in the foregoing experiments. Hoskins and McClure[23] estimated that the amount of adrenin secreted as a result of splanchnic stimulation was at first of this order. Elliott[24] found that the sensitiveness of the arrectores pilorum to adrenin varied with the functional use by each animal. In a similar manner it is possible that response of the blood vessels to adrenin is in accordance with their functional use in times of excitement, there being an active dilation of the peripheral arteries and simultaneously a constriction of the arteries of the splanchnic region. Such an arrangement would assure the motor organs an abundant blood supply for their most efficient action.

SUMMARY

1. Dilute adrenalin slowly injected caused a fall in general blood pressure in 48 out of 53 animals tried. Three of the animals in which a fall did not occur, were in poor physical condition and one was just recovering from the effects of alcohol.

2. Dilute adrenalin caused dilation of the peripheral arteries even after extremely low pressures had been produced by hemorrhage. The same dose of adrenalin caused constriction of the splanchnic arteries.

3. When the blood pressure was lowered by depressor stimulation the same dose of adrenalin caused a fall, a rise and fall or a pure rise depending somewhat upon the height of the pressure.

4. The average latent period for the peripheral fall in the blood pressure (from doses of 0.2 cc. adrenalin, 1: 100,000) was 15.7 seconds; the latent period for the splanchnic rise was 18.6 seconds. The duration of the splanchnic rise was 37.8 seconds, while the duration of the peripheral fall was 59 seconds.

5. The threshold for the change of a fall to a rise in the peripheral arteries, when adrenalin was injected (in three animals only) over a period of from ten to twenty seconds, was between 0.1 and 0.3 cc. of a 1: 10,000 solution.

[23] Hoskins and McClure: Arch., Internal Med., 1912, x, 352.

[24] Elliott: Loc cit., 416.

6. Large doses of ergotoxine phosphate inhibit the splanchnic response to dilute adrenalin. The existence of sympathetic vasodilator nerves in the peripheral arteries and their absence in the splanchnic arteries would account for the opposed action of like doses of dilute adrenalin upon the peripheral and splanchnic arteries.

I wish to thank Dr. W. B. Cannon for suggesting this research and for his advice and criticism.

THE OSMOTIC PROPERTIES OF CALCIUM AND MAGNESIUM PHOSPHATE IN RELATION TO THOSE OF LIVING CELLS

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INTRODUCTION

In 1867 Traube suggested that living cells might owe their semi-permeable properties, at least in part, to precipitates of inorganic substances.[1] In recent times this suggestion has been nearly lost sight of and the attention of physiologists has been chiefly centered on the question whether or not the semi-permeable surface of the cell is composed of lipoid.

Most of the work on this question has been directed toward discovering to what substances the cell surface is or is not permeable, it being tacitly assumed that we know or can predict *a priori* the semi-permeable properties of "lipoids." But the little work that has been done with artificial lipoid membranes is far from justifying this assumption.

It has been shown, for instance, by Nathansohn[2] and Ruhland[3] that artificial lipoid membranes, when soaked with water are quite permeable to substances which are soluble in water, whether or not the substances in question are soluble in lipoids. On the other hand, water-free lipoid membranes are impermeable to water as well as to dissolved substances.

It was shown by Pfeffer[4] that semi-permeable membranes can be made of calcium phosphate, and it is well known that both calcium and inorganic phosphates are present in most living cells. Since Pfeffer's time there has accumulated a considerable body of evidence which shows that calcium plays a very important part in the activities of living tissues, and particularly that the permeability of the cell surfaces is affected by its presence or absence.

[1] Traube: Arch. f. Anat. Physiol., und wissensch. Med.; 1867, xxxiv, 146.
[2] Nathansohn: Jahrb. f. wissensch. Bot., 1904, xxxix, 607.
[3] Ruhland: Jahrb. f. wissensch. Bot., 1909, xlvi, 1.
[4] Pfeffer: Osmotische Untersuchungen, Leipzig, 1877, 11.

Thus Loeb[5] finds that $CaCl_2$ can protect the fish, Fundulus, against the toxic effects of KCl. He gives reasons for thinking that the calcium protects by forming an insoluble compound with some "organic anion" at the surface of contact between the fish and the solution (Loc. cit., pp. 320 and 321).

Somewhat more direct in their bearing on this question perhaps are certain experiments of Osterhout on plants. This author gives a series of experiments which indicate that the surface of spirogyra filaments is decidedly more permeable to NaCl than to $CaCl_2$, and that the addition of $CaCl_2$ to an NaCl solution may prevent the latter salt from entering.[6] In another series of experiments he shows that the resistance of the leaf cells of the common kelp (laminaria) to the passage of an electric current may be increased by the action of $CaCl_2$, and gives reasons for thinking that the salt acts by decreasing the permeability of the plasma membrane.[7]

Both Loeb and Osterhout find that the effects of the $CaCl_2$ can be to a greater or less extent imitated by substituting for it certain other salts such as $SrCl_2$, $BaCl_2$, $MgCl_2$, and $La_2(NO_3)_6$. The kations of all these salts form insoluble compounds with the phosphates, and it seems, therefore, not impossible that they may all tend to decrease the permeability of the cells in the same way, namely by impregnating their surfaces with layers of insoluble phosphate.

Both calcium and magnesium are known to be present in most living cells. The physiological experiments make it appear that calcium is decidedly the more important of these two, but a consideration of the ash of certain tissues of which the permeability has been much studied, points to a different conclusion. Abderhalden, for instance, finds that the blood corpuscles of a number of the domesticated mammals contain small and rather variable quantities of Mg, but no Ca.[8] And Katz has shown that samples of striated muscle taken from the human being, pig, ox, calf, deer, rabbit, dog, cat, hen, frog, haddock, eel, and pike contain larger and more constant quantities of Mg than of Ca. The Mg content of the muscle in this series of animals varies from 0.017 per cent of the weight of the fresh tissue in the haddock to 0.037 per cent in the hen; while the Ca content varies from 0.002 per cent in the ox to 0.04 per cent in the pike.[9]

[5] Loeb: Biochem. Zeitschr., 1911, xxxii, 308.
[6] Osterhout: Science, N. S., 1911, xxxiv, 187.
[7] Osterhout: Science, N. S., 1912, xxxv, 112; ibid., xxxvi, 350.
[8] Abderhalden: Zeitschr. f. physiol. Chem., 1898, xxv, 106.
[9] Katz: Arch. f. d. gesammt. Physiol., 1896, lxiii, 1.

An interesting observation which indicates that magnesium phosphate is present in considerable quantities at the surfaces of the striated muscle fibers is recorded by Hürthle.[10] He finds that if fresh frog's muscle fibers are treated with ammonia, they "cover themselves" with crystals of magnesium ammonium phosphate. It is difficult to see how such crystals can be formed, unless magnesium phosphate is previously present.

In view of the facts given above, it has seemed to me worth while to make a study of the osmotic properties of calcium and magnesium phosphate. I had hoped to make a more or less extended comparison between the permeability of these precipitates to various substances and that of living cells. But some two years of work on this subject have convinced me that the time is not yet ripe for such a comparison. One begins by thinking of a semi-permeable membrane as a more or less simple and stable kind of filter by means of which water can readily be separated from most substances that can be dissolved in it. But this view is far from the truth. The studies that have been made on the copper ferrocyanide membrane, which is at present the best known semi-permeable membrane, show that its permeability varies greatly with the physical conditions under which it is formed and to which it is afterward subjected—with the electrical conditions at the time of its formation, with the time which elapses between its formation and the experiment, with temperature, with the presence or absence of electrolytes, and with the nature of the electrolytes which happen to be present.[11] If one wishes, therefore, to determine whether one osmotic membrane is more or less permeable to a given substance than another, one must be careful to control all these conditions. To do this in the case of artificial membranes formed in the laboratory is by no means easy; it is obviously impossible when one of the membranes to be studied is that of a living cell. The experiments to be reported, therefore, bear only in a general way on the question whether the semi-permeable properties of the phosphate membranes are similar to those of the surfaces of animal and plant cells. But they do throw some light on the semi-permeable properties of these precipitates and on certain fundamental questions regarding the nature of semi-permeable membranes and of osmotic reactions in general. I am compelled by external circumstances either to publish this work now or to lay it aside for an indefinite period, and these considerations impel me to publish it now, though the results are in many respects fragmentary and incomplete.

[10] Hürthle: Arch. f. d. gesammt. Physiol., 1903, C, 451.

[11] Morse: The osmotic pressure of aqueous solutions; Publication No. 198 of the Carnegie Institution of Washington, 1914, Chapter IV.

THE CONDITIONS ON WHICH DEPEND THE CRYSTALLIZATION OF CALCIUM AND MAGNESIUM PHOSPHATE

Morse believes that true semi-permeability is an attribute of colloids only, and gives many cogent reasons for holding this belief.[12] Experiences of my own, which will be described later, lead me to concur in this belief; and I have spent a considerable amount of time in inquiring under what circumstances the phosphates of calcium and magnesium fail to crystallize.

I have confined my inquiry to the orthophosphates and monohydrophosphates of the two metals, as the dihydrophosphates can exist only in acid solutions. Abegg describes the crystallization of both the orthophosphate [$Mg_3(PO_4)_2$] and monohydrophosphate [$MgHPO_4$] of magnesium and of the monohydrophosphate of calcium [$CaHPO_4$].[13] With regard to $Ca_3(PO_4)_2$, however, he says,[14] "This amorphous precipitate has never been observed to become crystalline."

$CaHPO_4$ precipitates were prepared by mixing together equimolecular solutions[15] of $CaCl_2$ and K_2HPO_4; $Ca_3(PO_4)_2$ precipitates, by mixing solutions of $CaCl_2$ with solutions of K_2HPO_4 to which KOH had previously been added. The KOH and K_2HPO_4 mixtures were made by adding together equal portions of equimolecular KOH and K_2HPO_4 solutions.

It was found that under these conditions the $CaHPO_4$ crystallized[16] within twenty-four hours, while the $Ca_3(PO_4)_2$ never crystallized, though its precipitates were kept sometimes for more than four months.

Precipitates of $MgHPO_4$ and $Mg_3(PO_4)_2$ were prepared in a manner corresponding to that which has just been described, $MgCl_2$ being substituted for the $CaCl_2$. The rapidity with which both these precipi-

[12] Morse: Loc. cit., pp. 87 and 209.

[13] Abegg: Handuch der anorganischen Chemie, Leipzig, 1905, vol. ii, Abt. 2, pp. 65 and 151.

[14] Ibid., p. 152.

[15] Concentrations are, throughout this article, given in the terms of what Morse calls "weight-normal solutions." See p. 479 of this article and "Osmotic pressure of aqueous solutions," Chapter V.

[16] It was determined whether or not the various precipitates crystallized by examining them microscopically. Such examination cannot, of course, show whether or not the precipitates are in a colloidal state in the sense of Morse, and capable of showing true semi-permeability. Morse finds, for instance, that precipitates of zinc ferrocyanide may become granular and lose their semi-permeable properties without becoming actually crystalline (Osmotic pressure of aqueous solutions, p. 91). But it may at least be said that crystalline precipitates do not exhibit semi-permeability.

tates crystallized was extremely variable. The $MgHPO_4$ sometimes crystallized in four days, and sometimes failed to crystallize at all, though its precipitates were kept for more than four months. I did not make any study of the conditions which control the crystallization of this precipitate, as it is so soluble that it can hardly be supposed to play a part in modifying the semi-permeable properties of living cells.

With regard to $Mg_3(PO_4)_2$ it was found that its crystallization depended on a number of conditions, of which the following were more or less extensively investigated. (1) Amount of alkali present. (2) Temperature. (3) Presence or absence of Ca. (4) Concentration of salts in supernatant fluid.

The influence of alkalinity on the crystallization of $Mg_3(PO_4)_2$ was investigated by mixing solutions of $MgCl_2$ and K_2HPO_4 together with varying quantities of KOH, and noting the time required for crystallization to occur (experiments 1 and 2). These experiments show that when varying proportions of $MgCl_2$, K_2HPO_4, and KOH are mixed together, the crystallization of the resulting precipitates depends on the relative proportions of the three ingredients. When the final mixture contains a higher molecular concentration of K_2HPO_4 than of KOH and enough Mg to combine with all the PO_4 as $Mg_3(PO_4)_2$, the resulting precipitates remain for the most part amorphous, for many weeks at least. But when the final mixture contains a higher molecular concentration of KOH than of K_2HPO_4 or not enough Mg to combine as $Mg_3(PO_4)_2$with all the PO_4 present, the resulting precipitate crystallizes more or less rapidly.

It is probably not correct to speak of the precipitates which have just been described as $Mg_3(PO_4)_2$. Such precipitates are probably mixtures of this compound with varying amounts of $MgHPO_4$ and $Mg(OH)_2$. There are many reasons for thinking that the quantities of the last two named substances present are small, but no special investigation of this question has been undertaken. For my purpose the main point of interest is the fact that magnesium phosphate combinations, which have a high degree of insolubility, may, under certain circumstances, remain amorphous indefinitely, or, at least, for a long time; for convenience the precipitates will in future be spoken of simply as *magnesium phosphate*.

Experiments 3 and 4 show the influence of temperature on the crystallization of magnesium phosphate. When formed above a certain critical temperature, which is not far from 23° the precipitates fail to crystallize, whereas they crystallize readily at lower temperatures. These

experiments indicate also that precipitates which have been formed at temperatures above 23° and have therefore failed to crystallize, show little or no tendency to crystallize, when later kept at lower temperatures. A comparison of experiment 3 with experiment 4 shows finally that, other things being equal, the precipitates have a greater tendency to crystallize when formed from more concentrated solutions. The precipitate formed from the less concentrated solutions at 20° in experiment 3 was only about half crystalline after 21 hours, while that formed from the more concentrated solutions in experiment 4 was completely crystalline at the end of the same period at the same temperature.

Experiment 5 shows that calcium has an inhibiting effect on the crystallization of magnesium phosphate precipitates.

THE OSMOTIC PROPERTIES OF CALCIUM AND MAGNESIUM PHOSPHATE AND OF COPPER FERROCYANIDE

The osmotic properties of calcium and magnesium phosphate and of copper ferrocyanide have been studied by making small, unsupported membranes of them according to the method of Traube[17], and by precipitating them on porous clay cups. The electrolytic method of precipitation of Morse[18] has not been employed in these experiments. It is, of course, highly desirable that the properties of phosphate membranes precipitated by the electrolytic method should be studied, but it would be out of the question to follow the technique of Morse in the rough preliminary survey which constitutes the subject matter of this article.

EXPERIMENTS WITH PHOSPHATE AND FERROCYANIDE MEMBRANES PRECIPITATED ON POROUS CLAY CUPS

Experimental methods

It has been my plan to determine as far as possible for what substances the phosphate membranes are impermeable and for what substances they are permeable, and to compare the rapidities with which substances of the latter class diffused through the membranes. To do this, it is not necessary to measure the osmotic pressure of the solutions used. The substance, of which the osmotic properties are to be studied,

[17] Traube: Arch. f. Anat., Physiol., und wissensch. Med., 1867, xxxiv, 123, 133, et seq.

[18] Morse: Loc. cit., 83–84.

may be precipitated on the inner surface of a porous cup, which may then be provided with suitable attachments for determining how rapidly fluid passes through it to the interior against a slight hydrostatic pressure. The cup is then filled with a solution of known composition and concentration and immersed for a given period in distilled water or in another known solution. At the end of the experimental period it is determined how much fluid has passed through the walls of the cup and the membrane; and the fluids within and without the cup are analyzed to determine how much, if any, of the solute experimented with has passed through the membrane from within the cup to the exterior.

I supposed at first that almost any kind of porous earthenware vessel would do for experiments of this class, and I spent a good deal of time in attempting to precipitate phosphate membranes and membranes of $Cu_2Fe(CN)_6$ on alundum filters and on various other kinds of porous clay vessels which allow a ready passage of water through their walls, but are supposed to hold back undissolved solids. I found, however, that all of the precipitates mentioned are easily forced through the walls of most of these vessels under pressures of a metre of water or less.

The porous cups which I finally found satisfactory were furnished me through the kindness of Prof. B. E. Livingston of the Johns Hopkins University. They are the regular cylindrical atmometer cups supplied by the *Plant World*, Tucson, Arizona; and are formed by pouring liquid kaolin and quartz mixture into dry plaster of paris moulds, and subsequently drying and burning the cups so formed. These cups are about 13 cm. high with an inner diameter of about 2.2 cm. and entirely unglazed. For the purposes of my experiments I filled the pores of the upper rims (about 2 cm. in height) with paraffin. This left an inner surface of about 70 sq. cm. on which the membrane was precipitated. The walls of the cup are about 0.25 cm. thick. These cups were connected by means of rubber attachments to an upper piece of glass provided with a stop-cock and an upright glass tube about one metre high. The whole cell so formed held from 70 to 80 cc. of fluid. The amount of fluid passing through the walls of the cup and the semi-permeable membrane was determined by noting the rise or fall of the meniscus in the upright tube. I used rubber attachments instead of the sealing wax used by Pfeffer[19] in his experi-

[19] Pfeffer: Osmotische Untersuchungen, Leipzig, 1877, 5 et seq.

ments, because I wished to work with alkalies in many cases, and because it was not necessary for me to make arrangements to withstand any considerable pressure. I need not describe my attachments in detail. They are such as will readily suggest themselves to anyone seriously interested in the subject. But it may be worth while to say that I found it much harder than I had supposed to entirely prevent leakage through rubber attachments, and overcame this difficulty only by using pretty tight fittings and binding the joints tightly with stout cotton thread which had previously been wet.

I removed the air from the porous cups by allowing first boiled distilled water and then a solution of one of the membrane-formers to seep through them under a pressure of about 70 cm. of water. Each of these fluids was allowed to seep through for a period of three or four days. After the cup had been thoroughly infiltrated with the outer membrane-forming solution, it was emptied of this fluid, rinsed out quickly with distilled water, dried slightly, filled with the inner membrane-forming solution and allowed to stand for twenty-four to forty-eight hours in the outer membrane-forming solution, there being no difference in the pressures within and without the cup. Subsequently a pressure of about 70 cm. of water was put on the inner membrane-forming solution, and the cup was allowed to stand for another twenty-four to forty-eight hours in the outer solution. In this way the semi-permeable membrane was precipitated on its inner surface.[20]

RESULTS OF THE EXPERIMENTS

I had little difficulty in making by these methods membranes of $Cu_2Fe(CN)_6$ which showed osmotic activity. Experiment 6 shows the behavior of such a membrane under various conditions, and experiment 7 gives the results of somewhat similar procedures with another copper ferrocyanide membrane.

These two experiments add a little to the already existing evidence for the view that the $Cu_2Fe(CN)_6$ membrane is impermeable for sugar[21] and highly permeable for NaCl.[22] Perhaps their most interesting result, however, is the rapid osmotic intake of fluid caused by the NaCl solution in spite of the facts that its osmotic pressure (as calculated from freezing point determinations) was nearly the same as that of

[20] Compare Pfeffer: Osmotische Untersuchungen, Leipzig, 1877, 4 et seq.

[21] Morse: Loc. cit., pp. 92 and 93.

[22] Traube: Arch. f. Anat. Physiol. und wissensch., Med., 1867, xxxiv, 137-141.

the sugar solution used, and that it escaped so rapidly from inside the cell to the exterior. In the first experiment the osmotic intake caused by the NaCl was nearly twice as rapid as that caused by the sugar; and, as the NaCl period fell between two sugar periods in which the osmotic intake was nearly the same, it can hardly be supposed that the result was due to any irreversible change taking place in the membrane. In the second experiment the osmotic intake caused by the NaCl was slower than that caused by the sugar, but it seems to me reasonable to explain this as caused by the decidedly greater leakiness of the second membrane.

For the experiments with membranes of $Ca_3(PO_4)_2$ the cups were provided with the same fittings as in the experiments which have just been described, and subjected to the same preliminary treatment. They were then impregnated with a 0.09M $CaCl_2$ solution and filled with a 0.075M K_2HPO_4 + 0.075M KOH solution, the details of the procedure being the same as in the case of the ferrocyanide membrane. Experiments 8 and 9 show that semi-permeable membranes can be made of $Ca_3(PO_4)_2$, and give an idea of the permeability of this membrane with respect to cane sugar and potassium hydroxide. The membrane is impermeable to cane sugar, but quite permeable to potassium hydroxide.

An interesting result of experiment 8 is shown in the period from March 13 to March 24. In the first four days of this period the cell contained a 0.22M $C_{12}H_{22}O_{11}$ solution; in the next three days, a KOH solution of about the same calculated osmotic pressure; and in the last four days, the same sugar solution as at first. It was immersed during the whole period in a 0.009M $CaCl_2$ solution. The cell showed considerable osmotic activity in both the first and second sugar periods, fluid passing to the interior against a moderate hydrostatic pressure. But during the alkali period there was practically no osmotic activity after the first hour, in spite of the fact that the alkali solution had about the same calculated osmotic pressure as the sugar solution. It is true that the alkali escaped fairly rapidly from the cell to the exterior. But the rate of its escape was not many times greater than that of the NaCl in experiment 6 between the dates March 16 and 20, and yet the NaCl solution caused a more rapid intake of fluid in this case than did a sugar solution of the same calculated osmotic pressure. Further, the results obtained in experiment 11 between November 3 and 11 show that a sodium chloride solution may cause greater osmotic activity in a mag-

nesium phosphate membrane than a sugar solution of the same calculated osmotic pressure, in spite of the fact that in this case also the salt escapes to a considerable extent through the membrane while the sugar does not. KOH does not cause the crystallization of the $Ca_3(PO_4)_2$ membrane, nor does it produce any known chemical change in it; the membrane is formed in the presence of a pretty strongly alkaline solution. Further, the fact that the membrane showed decided osmotic activity in the presence of a sugar solution subsequent to its treatment with the alkali indicates that this latter produced no destructive irreversible change in it. It seems likely, therefore, that alkalies and salts produce entirely different osmotic responses when applied to the surface of a semi-permeable membrane. It would be easy to find some explanation for this difference if it may be supposed that all such membranes are colloidal in nature, but difficult on any other basis.

Experiment 9 shows the same sort of result with sugar on the $Ca_3(PO_4)_2$ membrane as experiment 8; and, in addition, the result of filling the cell with a mixture of KCl and KOH. A rather slow intake of fluid is produced. Several features of the alkali effects shown in experiment 8 are reproduced, but it would be rash to attempt a detailed interpretation of these results at present.

Experiments 10, 11, and 12 show some of the properties of the magnesium phosphate membrane; the general method of making these membranes was the same as in the case of the ferrocyanide and calcium phosphate membranes. These experiments show that magnesium phosphate may retain its osmotic activity for two months or more in the absence of its membrane formers; that it is quite impermeable to cane sugar; somewhat permeable to KCl and NaCl; and apparently about equally so to each of the two salts. It seems to be decidedly less permeable to either NaCl or KCl than is the copper ferrocyanide membrane. It is highly permeable to ethyl alcohol.

In addition, two interesting points come out which bear on the nature of osmotic action in general. In experiment 11 the results obtained between November 3 and 11 indicate that a sodium chloride solution sets up a greater osmotic activity in the magnesium phosphate membrane than does a cane sugar solution of the same calculated osmotic pressure, in spite of the fact that the salt escapes through the membrane fairly rapidly and the sugar not at all. This result is similar to that obtained in experiment 6 in the case of the $Cu_2Fe(CN)_6$ membrane (see page 464); it indicates that solutions of neutral electrolytes have the

general property of setting up a more rapid osmotic action in semi-permeable membranes than solutions of non-electrolytes of the same calculated osmotic pressure.

The results obtained in experiment 11 from November 3 to 7, 11 to 20, 1914, and from November 25, 1914 to February 12, 1915 indicate that the rapidity with which NaCl escapes through the magnesium phosphate membrane depends, among other things, on the concentration of the outer solution. During these periods the salt uniformly escaped more rapidly when the outer solution was more dilute.

In regard to the experiments on the osmotic properties of magnesium phosphate, it must be added that there were a number of other attempts to precipitate semi-permeable membranes of this substance on porous cups, besides the three which are described in the protocols. In some of these attempts the precipitations were carried out at temperatures below 23°; in others, the proportions of alkali added to the phosphate solutions were such as would induce crystallization of the magnesium precipitates. It was found that the precipitates formed under these conditions showed no osmotic activity. The experiments with magnesium phosphate taken all together, therefore, are in accord with the view that precipitates must be in a colloidal condition if they are to exhibit osmotic acitivty.

EXPERIMENTS WITH UNSUPPORTED MEMBRANES

Traube studied the osmotic properties of a number of substances by precipitating thin layers of them across the mouths of small glass tubes. He filled tubes, for instance, with solutions of $K_4Fe(CN)_6$, and immersed the lower open ends in solutions of copper salts. Under these circumstances a layer of $Cu_2Fe(CN)_6$ forms across the open mouth of the tube. I have attempted to form magnesium phosphate membranes in a similar way, but without any very satisfactory results. If a small glass tube be filled with a solution of K_2HPO_4 and KOH and dipped into a solution of $MgCl_2$, a layer of magnesium phosphate is, of course, precipitated across its mouth. But this layer rapidly becomes thicker, and the least disturbance suffices to detach it from the walls of the tube; so that it cannot be used even for the rough kind of osmotic experiment described by Traube.

Membranes of $Ca_3(PO_4)_2$, however, produced in this way are quite as resistant and satisfactory for experimentation as the $Cu_2Fe(CN)_6$ membranes. To work with such membranes it is necessary in the first

place to use a rather small glass tube. Further, the phosphate and calcium solutions used in making the membrane must have somewhere near the same osmotic pressure; otherwise, the membrane will be very soft, and will rapidly become thicker.

In my experiments I have used tubes which had an inner cross sectional area of about 0.14 sq. cm. (inner diameter, about 0.42 cm.). These were closed at the top with a rubber tube and pinch cock; the lower ends were inserted through bored corks into small cylindrical vials.[23] In some experiments a few cubic centimeters of 0.186M K_2HPO_4 + 0.186M KOH[24] were placed in the vials and the lower ends of the tubes were filled with 0.666M $CaCl_2$ solution. Under these circumstances there is a considerable tendency for fluid to pass through the membrane from the phosphate to the calcium solution. As the calcium solution has a decidedly higher calculated osmotic pressure than the phosphate solution, there can be little doubt that this phenomenon is the result of osmotic action on the part of the calcium phosphate membrane.

It is perhaps not generally realized by biologists that impermeability to NaCl and KCl is by no means a usual characteristic of semi-permeable membranes. Traube[25] asserts that a $Cu_2Fe(CN)_6$ membrane when infiltrated with AgCl becomes impermeable to KCl. But he finds the $Cu_2Fe(CN)_6$ membrane by itself highly permeable to KCl and probably also to NaCl (loc. cit., pp. 137–141). Morse[26] describes experiments in which KCl solutions were allowed to act on electrolytically deposited $Cu_2Fe(CN)_6$ membranes. He found that old (and probably thick) membranes at first exhibited a high resistance to the passage of KCl, but that the salt had a tendency to render the membrane permeable to itself.

In experiments of my own I have found the $Cu_2Fe(CN)_6$ membrane highly permeable both to KCl and to NaCl. In order to compare the permeability of this membrane to NaCl with that of the $Ca_3(PO_4)_2$ membrane, the following experimental procedure was employed. Four vials, and four glass tubes with inner diameters of 0.42 cm. were arranged as described above. Into the lower end of each of the tubes was drawn 0.28 cc. of 0.125M $Na_4Fe(CN)_6$ + 0.5M NaCl solution;

[23] Compare Traube: Arch. f. Anat., Physiol. u. wiss. Med., 1867, xxxiv, 123 and 133.

[24] Made up by adding 32.4 grams K_2HPO_4 and 10.4 grams KOH to liter of water.

[25] Traube: Loc. cit., p. 146.

[26] Morse: Loc. cit., pp. 211, et seq.

and 10 cc. of 0.25M $CuSO_4$ solution was placed in each of the vials. The lower ends of the tubes containing the chloride and ferrocyanide mixture were then immersed in the copper solution, and 0.14 cc. of the chloride and ferrocyanide mixture was forced through the lower end of the tube and appeared below it as a drop covered by the $Cu_2Fe(CN)_6$ membrane. This drop was then drawn back into the tube carrying the membrane behind it and finally leaving the latter in a much folded state across the tube's mouth.[27] The tubes were then immediately withdrawn from the vials and the solution left in the latter was analyzed for Cl. The amount found was taken to represent that which escaped from the inner solution during the formation of the membranes. The same procedure was then exactly repeated up to the point at which the drop of chloride and ferrocyanide mixture was drawn back into the tubes, leaving the folded membrane across its mouth. At this point the tubes, instead of being withdrawn, were left for twenty minutes with their lower ends immersed in the $CuSO_4$ solution. As the chloride and ferrocyanide mixture has a decidedly higher osmotic pressure than the $CuSO_4$ solution, fluid passed from the latter to the former during this period, more rapidly at first and more slowly afterward; at the end of the twenty minutes the membrane was completely distended and filled with a drop of diluted chloride and ferrocyanide mixture, about 0.14 cc. of fluid having passed through it during the interval. The drops of chloride and ferrocyanide mixture were now again drawn back into the tubes, the tubes were withdrawn from the $CuSO_4$ solution, and this last was analyzed for Cl. In both cases, of course, the Cl must represent NaCl which has escaped from the chloride and ferrocyanide mixture.

It was found in the first case when practically no time was allowed for diffusion that the 40 cc. of $CuSO_4$ solution contained 0.0015 gram of NaCl. In the second case, after the diffusion had gone on for twenty minutes the 40 cc. of $CuSO_4$ solution contained 0.0115 gram of NaCl. It may be supposed that 0.0115 minus 0.0015 or 0.01 gram of NaCl diffused through the four $Cu_2Fe(CN)_6$ membranes in the course of twenty minutes. The four glass tubes contained originally 0.28×4 or 1.12 cc., of 0.125M $Na_4Fe(CN)_6$ + 0.5M NaCl solution or 0.03248 gram of NaCl. Of this 0.0015 gram was lost during the formation of the membrane, leaving 0.031 gram at the time the diffusion began.

[27] Compare Traube: Arch. f. Anat., Physiol. u. wiss. Med., 1867, xxxiv, pp. 136 and 137.

And of this 0.031 gram 0.01 gram or about 32 per cent diffused through the four membranes in the course of twenty minutes.

A similar experiment was carried out to test the permeability of the $Ca_3(PO_4)_2$ membrane to NaCl. In this case the four tubes were filled each with 0.28 cc. of 0.24M $CaCl_2$ + 0.375M NaCl solution and immersed in 10 cc. of 0.186M K_2HPO_4 + 0.186M KOH solution. It was found that under these circumstances it required about two hours for as much fluid to pass through the $Ca_3(PO_4)_2$ membranes as passed through the $Cu_2Fe(CN)_6$ membranes in the previously described experiment in twenty minutes. In this case, therefore, the diffusion was allowed to go on for two hours instead of for twenty minutes; otherwise the procedure was the same as in the previously described experiment.

When practically no time was allowed for diffusion the 40 cc. of 0.186M K_2HPO_4 + 0.186M KOH solution contained 0.0021 gram NaCl. When the diffusion was allowed to go on for two hours, the 40 cc. of alkaline phosphate solution contained 0.0034 gram NaCl. It may be supposed therefore that 0.0013 gram of NaCl diffused through the four $Ca_3(PO_4)_2$ membranes in the course of two hours. The four tubes of 0.24M $CaCl_2$ + 0.375M NaCl solution contained originally 0.0243 gram of NaCl, and of this 0.0021 gram was lost during the formation of the membranes leaving 0.0222 gram at the time the diffusion began. 0.0013 is about 5.8 per cent of 0.0222, and it appears, therefore, that only about 5.8 per cent of the NaCl contained in the four tubes diffused through the $Ca_3(PO_4)_2$ membranes in the course of two hours. That is about five times as much NaCl diffused through the $Cu_2Fe(CN)_6$ membrane in twenty minutes as diffused through the $Ca_3(PO_4)_2$ membrane under more or less similar conditions in two hours. I have confirmed these results by other experiments which it is not necessary to describe in detail, and I am prepared to assert, therefore, that, under the conditions of formation which have been described, the $Cu_2Fe(CN)_6$ membrane is much more permeable to NaCl than the $Ca_3(PO_4)_2$ membrane.

PROPERTIES OF THE CELLOIDIN MEMBRANE

In my earliest experiments I used celloidin membranes on which layers of calcium phosphate were precipitated. Celloidin or collodion membranes have been used for many years for the study of osmotic phenomena; Smith[28] has suggested their use as a basis for the precipi-

[28] Smith: Science, 1913, N. S., xxxvii, 379.

tation of other forms of semi-permeable material. Since Traube's time it has been known that the permeability of sheets of celloidin varied with the circumstances of their formation. Such sheets are formed by pouring an alcohol-ether solution of celloidin on a flat surface, allowing the alcohol and ether to evaporate to a certain extent, and then immersing the sheet so formed in water. If the water be applied before much of the alcohol and ether have evaporated, the membrane will be found highly permeable; otherwise, it becomes quite impermeable to water as well as to dissolved substances.[29]

I formed my celloidin membranes by pouring the alcohol-ether solution over the inner surface of a beaker, and carried out several experiments to determine the properties of the membranes so formed under different conditions of drying. The celloidin sacs were tied around large rubber corks, and the cells so formed were fitted with long upright outlet tubes, filled with various solutions, and immersed in others. When the celloidin membrane was immersed in water less than an hour after its formation it was highly permeable to water and dissolved salts; it was easy to show that a solution of K_2HPO_4 could be rapidly forced through its walls by the very moderate hydrostatic pressure of 50 to 100 cm. of water. But if the membrane was not wet with water until after the alcohol-ether mixture had dried out for twenty-four hours or more, it became highly impermeable, though it showed some osmotic activity. The following experiment shows this. A celloidin membrane was allowed to dry for twenty-four hours, and then washed for twenty-four hours in tap water. At the end of this time it was used in making a cell, which was filled with 0.075M K_2HPO_4 and immersed in distilled water. The contents of the cell were put under a pressure of 78 cm. of water. The meniscus immediately began to rise in the outlet tube and continued to do so steadily for the twenty days during which the experiment was continued at a rate indicating that a little more than 0.1 cc. of fluid per day was passing into the cell.[30] At the end of the twenty days the water in which the cell had been immersed was tested for K_2HPO_4 and found to contain none.[31] In another similar experiment, similar results were obtained.

In still another experiment a membrane was used which had been

[29] See Traube: Arch. f. Anat. Physiol., und wissensch. Med., 1867, xxxiv, 106

[30] The area of semi-permeable surface was involved in this experiment was about 60 sq. cm.

[31] The escape of 1 per cent of the K_2HPO_4 originally contained in the cell could easily have been detected by means of the test used.

dried for somewhere between one and twenty-four hours before being wet with water. The cell made with this membrane was filled with 0.075M K_2HPO_4 solution and immersed in distilled water. The meniscus immediately began to rise in the outlet tube, indicating a rapid intake of fluid; about 2 cc. passed through the membrane[30] into the cell in the first half hour. In the next two hours only about 1.5 cc. of fluid passed into the cell, and at the end of that time a good deal of K_2HPO_4 was found to have escaped to the distilled water surrounding it.

These experiments seem to me interesting, because they show so clearly that a given chemical substance may have enormously different semi-permeable properties under different physical conditions. It was found possible to alter the osmotic properties of the celloidin membrane by precipitating calcium phosphate on it; but, as it was not possible accurately to control the variable osmotic properties of the celloidin itself, the experiments carried out along these lines are of very doubtful significance, and I do not consider it worth while to give any description of them.

DISCUSSION

The colloidal character of semi-permeable membranes and the nature of osmotic action

The results of the experiments described in the preceding pages lend support to the two hypotheses advanced by Morse—the hypotheses, namely, that semi-permeable membranes are always colloidal, and that the passage of water through such membranes is the result of unequal hydration at the opposite surfaces of the colloid.

The first of these hypotheses is supported by the facts that a membrane highly impermeable to K_2HPO_4 and yet showing some osmotic activity can be made of an undoubted colloid, celloidin (pp. 469–471); that semi-permeable membranes can readily be made of $Ca_3(PO_4)_2$, which never crystallizes (p. 459 and pp. 464, 465) that semi-permeable membranes can be made from magnesium phosphate only under such conditions as prevent its crystallization (p. 466); and that the semi-permeable properties of the various membranes are altered by such influences as would be likely to change the physical state of colloids (pp. 464–466, and pp. 469–471). Finally this first hypothesis is supported by whatever evidence tends to support the second, for the view that osmotic action depends on unequal hydration at the two surfaces of a colloid membrane presupposes the existence of a colloid membrane to start with.

The hypothesis that the passage of water through a semi-permeable membrane is the result of unequal hydration of the two surfaces is supported by the anomalous results obtained with regard to the amount of osmotic activity set up by salt, sugar, and potassium hydroxide solutions. A sodium chloride solution causes a more rapid passage of water through the ferrocyanide and magnesium phosphate membranes than does a cane sugar solution of the same calculated osmotic pressure, while an equally concentrated potassium hydroxide solution causes practically no osmotic activity in the calcium phosphate membrane. The salt and alkali escape through the membranes with considerable rapidity, while the sugar does not escape at all (pp. 464–466). If the hypothesis just stated is correct, these facts might be explained as the result of the well known rule that neutral electrolytes have a greater dehydrating effect on colloids than non-electrolytes while alkalies tend to increase their power of holding water; I do not see at present how they can be explained on any other basis.

Certain experiments reported many years ago by Graham[32] are interesting in this connection. Graham found that the osmotic activity set up in a piece of pig's bladder by sugar solutions was very small in comparison to that set up by salt solutions, and that when the bladder separated a dilute solution of acid from distilled water, a considerable quantity of fluid passed through the membrane from the acid solution to the other side. Graham's results with the sugar and salt solutions are similar to mine, though not so clear cut, because the salt solutions used by him had a much higher calculated osmotic pressure than the sugar solutions. The result with the acid, however, is very striking. It seems quite inexplicable from the ordinary conceptions of osmosis and osmotic pressure, but can be readily explained on the hydration hypothesis from the well known fact that acids tend to hydrate colloids even more strongly than pure water.

If semi-permeable membranes are really hydrated colloids, it seems probable that their semi-permeable properties would vary with the degree of their hydration. My experiments furnish evidence for the view that this is the case, and also for the view that the membranes become more readily permeable both to water and dissolved substances the greater the degree of their hydration. Perhaps the most direct evidence for this view is the series of experiments with celloidin membranes

[32] Quoted by Girard: Journ. d. Physiol. et de path. générale, 1911, xiii, pp. 365–367.

(pp.469–471). But on p.466 it is shown that the magnesium phosphate membrane also is more permeable to salts when it is in contact with a more dilute outer solution and, therefore, presumably in a state of greater hydration.

Much of the peculiar behavior of living cells would find a ready explanation from the considerations above set forth and from the additional consideration that in the case of living cells we are dealing probably always with leaky membranes. The experiments of Morse on the osmotic pressure of potassium chloride and of lithium chloride[33] show that we have yet much to learn regarding the osmotic behavior of electrolytes even with respect to the most ideal semi-permeable membranes that have yet been produced. The conditions become much more complicated; and the results, correspondingly less predictable, when we have to deal with such membranes as may be supposed to cover the surfaces of living cells.

DO MAGNESIUM AND CALCIUM PHOSPHATE PLAY ANY PART IN THE OSMOTIC PROPERTIES OF LIVING TISSUES?

It is well known that calcium, magnesium, and inorganic phosphates are constant or nearly constant constituents of living tissues; and there is much evidence (reviewed at the beginning of this article) for the view that calcium, at least, plays a very important physiological role, and has the property of rendering the surfaces of cells less permeable for other ions. The experiments reported in this article are at least sufficient to show that both calcium and magnesium phosphate are capable of forming semi-permeable membranes.

Beyond this, however, the experiments do not go very far in showing either that these substances do or do not play an important part in giving to living cells their semi-permeable properties. It is hardly possible that they should, because we know at present so little, on the one hand, of the influences which affect the permeability of osmotic membranes, and, on the other, of the conditions which obtain in living cells. Still, it seems worth while to review very briefly those of the results which bear on this question.

Both the calcium and magnesium phosphate membranes are highly impermeable to cane sugar, potassium phosphate, calcium chloride, and magnesium chloride. Under certain conditions they may show a con-

[33] Morse: Loc. cit., Chapter XI.

siderable degree of impermeability to sodium chloride and potassium chloride. The calcium phosphate membrane is permeable to potassium hydroxide, when that substance is present in considerable quantities. The magnesium phosphate membrane is quite permeable to ethyl alcohol.

The surfaces of living cells are commonly more or less impermeable to cane sugar and to the salts mentioned above. They are always, so far as I know, quite permeable to ethyl alcohol. Most cells are killed by potassium hydroxide when it is present in any considerable quantity, and, at the same time, their surfaces become permeable to it as well as to crystalloids generally. Striated muscle cells and kidney cells are apparently decidedly more permeable to potassium chloride than to sodium chloride.[34] In this respect they seem to differ from the magnesium phosphate cells. But the surfaces of red blood corpuscles are equally impermeable to both these salts.[35] These facts and a great many others[36] show how complicated is the question of the permeability of living cells.

It is perhaps just to close this part of the discussion by saying that the semi-permeable properties of magnesium and calcium phosphate, so far as they have been studied, are as much like those of living tissues as are the semi-permeable properties of any other artificial osmotic membranes that have been examined up to this time.

In conclusion I wish to extend my most heartfelt thanks to Dr. John Marshall and to the staff of the Hare Chemical Laboratory of the University of Pennsylvania, without whose kind assistance and support the work reported in the foregoing article would have been difficult or impossible.

SUMMARY

1. Semi-permeable membranes can be formed both from calcium and from magnesium phosphate.

2. In order to form semi-permeable membranes from magnesium phosphate, it is necessary to precipitate it under such conditions that it does not crystallize.

[34] Siebeck: Arch. f. d. gesammt. Physiol., 1912, cxlviii, 443; 1914, cl, 316. Meigs: Journ. Exper. Zool., 1912, xiii, pp. 518 520.

[35] Hamburger: Osmotischer Druck und Ionenlehre, Wiesbaden, 1902, vol. 1, pp. 208 and 209.

[36] The surfaces of human red blood cells and of these of certain other animals, for instance, appear to be quite permeable to dextrose; see Kozawa: Biochem. Zeitschr., 1914, lx, 231; Masing: Arch. f. d. gesammt. Physiol., 1914, clix, 476.

3. The calcium phosphate membrane is impermeable or nearly impermeable to cane sugar, dipotassium phosphate and calcium chloride, only slightly permeable to sodium chloride, and quite permeable to potassium hydroxide.

4. The magnesium phosphate membrane is impermeable or nearly impermeable to cane sugar, dipotassium phosphate and magnesium chloride, somewhat permeable to sodium and potassium chloride, and highly permeable to ethyl alcohol.

5. Anomalous results are obtained when the calcium phosphate, magnesium phosphate, and copper ferrocyanide membranes are subjected to the action of neutral electrolytes and alkalies. Sodium chloride solutions cause a more rapid osmotic action in the copper ferrocyanide and magnesium phosphate membranes than do cane sugar solutions with the same calculated osmotic pressure, in spite of the fact that the salt escapes through the membrane to a considerable extent, while the sugar does not. Potassium hydroxide produces no lasting osmotic activity in the calcium phosphate membrane, though it apparently causes no marked irreversible change in the membrane, and does not escape through it very much faster than does sodium chloride through the copper ferrocyanide membrane.

6. It is shown that the semi-permeable properties of celloidin membranes depend to a very great extent on their physical state, and reasons are given for thinking that this is true of semi-permeable membranes in general.

7. Evidence, in addition to that already given by Morse, is adduced for the view that semi-permeable membranes are always colloidal, and that the passage of water through them is the result of unequal hydration of the colloid at its two surfaces.

Experiment 1

12.05 to 12.30 p.m., April 30, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 8.4 cc. 0.267M $MgCl_2$	12.05 to 12.30 p.m., April 30, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 8.4 cc. 0.267M $MgCl_2$ and 0.8 cc. 0.86M KOH	12.05 to 12.30 p.m., April 30, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 8.4 cc. 0.267M $MgCl_2$ and 1.6 cc. 0.86M KOH	12.05 to 12.30 p.m., April 30, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 8.4 cc. 0.267M $MgCl_2$ and 2.0 cc. 0.86M KOH
12.05 p.m., May 2. Precipitate is now almost entirely crystalline	12.15 p.m., May 2. Precipitate contains a few crystals, but is still for the most part amorphous	12.15 p.m., May 2. Precipitate is still entirely amorphous	12.15 p.m., May 2. Precipitate is now chiefly made up of crystals
11.40 a.m., June 6. Precipitate now entirely crystalline.	11.50 a.m., June 6. Precipitate contains a good many crystals, but is still, for the most part, amorphous	12.00 m., June 6. Precipitate still entirely amorphous	12.00 m., June 6. Precipitate now almost entirely crystalline

Experiment 2

2.53 p.m., Oct. 14, 1914. 10 cc. 0.15M K_2HPO_4 mixed with 5 cc. 0.2M $MgCl_2$ and 0.5 cc. 0.86M KOH	3.00 p.m., Oct. 14, 1914. 5 cc. 0.15M K_2HPO_4 mixed with 10 cc. 0.2M $MgCl_2$ and 0.5 cc. 0.86M KOH
3.55. Precipitate contains a few small crystals, but is still, for the most part, amorphous	4.00. Precipitate still entirely amorphous.
10.30 a.m., Oct. 15. Precipitate is still largely amorphous but contains a good many crystals	10.35 a.m., Oct. 15. Precipitate still entirely amorphous.
11.15 a.m., Oct. 16. Precipitate is now about half crystalline and half amorphous	11.15 a.m., Oct. 16. Precipitate still entirely amorphous

Experiment 3

3.40 p.m., Dec 4, 1914. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 10°	4.10 p.m., Dec. 1, 1914. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096 M KOH. Temp. 18°	12.45 p.m., Dec. 10, 1914. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 20°	11.50 a.m., Jan. 23, 1915. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 23°	2.54 p.m. Dec. 11, 1914. 5 cc. 0.3M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 27°
11.27 a.m., Dec. 5. Precipitate entirely crystalline. Temp. 10°	10.10 a.m. Dec. 2. Precipitate entirely crystalline. Temp. 18°	9.25 a.m., Dec. 11. Precipitate about half crystalline and half amorphous. Temp. 20°.		10.30 a.m. Dec. 12, Precipitate still entirely amorphous. Temp. 21.5°
		10.20 a.m., Dec. 12. Precipitate is about two - thirds crystalline and one-third amorphous. Temp. 19°	9.30 a.m., Jan. 25. Precipitate contains a fair number of crystals, but is still, for the most part, amorphous. Temp. 14°	
				11.47 a.m. Dec. 15, Precipitate still entirely amorphous. Temp. 15°
		12.30 p.m., Feb. 19, 1915. Precipitate now entirely crystalline. Temp. 21° (1)	2.30 p.m., Feb. 19, 1915. Precipitate contains a good many crystals, but is still, for the most part, amorphous. Temp. 21° (1)	12.47 p.m. Feb. 19, 1915. Precipitate contains a few crystals, but is still, for the most part, amorphous. Temp. 21° (1)
		(1) Between Dec. 10, 1914, and Feb. 19, 1915, the temperature varied between 10° and 25°	(1) Between Jan. 25 and Feb. 19 the temperature varied between 10° and 25°	(1) Between Dec. 12, 1914 and Feb. 19, 1915 the temperature varied between 10° and 25°

Experiment 4

3.40 p.m., Dec. 4, 1915. 5 cc. 0.5M $MgCl_2$ mixed with 5 cc. 0.2M K_2HPO_4 + 0.14M KOH. Temp. 10°	12.45 p.m., Dec. 10, 1914. 5 cc. 0.5M $MgCl_2$ mixed with 5 cc. 0.2M K_2HPO_4 + 0.14M KOH. Temp. 20°	11.55 a.m., Jan. 23, 1915. 5 cc. 0.5M $MgCl_2$ mixed with 5 cc. 0.2M K_2HPO_4 + 0.14M KOH. Temp. 23°	2.54 p.m., Dec. 11, 1914. 5 cc. 0.5M $MgCl_2$ mixed with 5 cc. 0.2M K_2HPO_4 + 0.14M KOH. Temp. 27°
11.35 a.m., Dec 5. Precipitate entirely crystalline. Temp. 10°	9.30 a.m., Dec. 11. Precipitate entirely crystalline. Temp. 20°		10.32 a.m., Dec. 12. Precipitate still entirely amorphous. Temp. 21.5°
		10.10 a.m., Jan. 25. Precipitate contains a large number of crystals, but is still chiefly amorphous. Temp. 14°	
			11.53 a.m., Dec. 15. Precipitate still entirely amorphous. Temp. 15°
		2.33 p.m., Feb. 19, 1915. Precipitate contains a large number of crystals, but is still chiefly amorphous. Temp. 21° (1)	12.50 p.m., Feb. 19, 1915. Precipitate contains a few crystals but is still chiefly amorphous. Temp. 21° (1)
		(1) Between Jan. 23 and Feb. 19 the temperature has varied between 10° and 25°	(1) Between Dec. 12, 1914 and Feb. 19, 1915, the temperature has varied between 10° and 25°

Experiment 5

12.00–12.05 p.m., Dec. 7, 1914. 5 cc. 0.2M $MgCl_2$ mixed with 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 20°	12.00–12.05 p.m., Dec. 7, 1914. 5.15 cc. 0.19M $MgCl_2$ + 0.019M $CaCl_2$ mixed with 5.15 cc. 0.134M K_2HPO_4+0.096M KOH. Temp. 20°	12.00–12.05 p.m., Dec. 7, 1914. 5.8 cc. 0.17M $MgCl_2$ + 0.086M $CaCl_2$ + 5 cc. 0.134M K_2HPO_4 + 0.096M KOH. Temp. 20°
3.15 p.m. Precipitate entirely crystalline. Temp. 21°	3.22 p.m. Precipitate about half crystalline and half amorphous. Temp. 21°	3.25 p.m. Precipitate entirely amorphous. Temp. 21°
	10.00 a.m., Dec. 8. Precipitate about half crystalline and half amorphous. Temp. 17°	10.05 a.m., Dec. 8. Precipitate contains a very few crystals, but is still almost entirely amorphous. Temp. 17°
	2.20 p.m., Feb. 19, 1915. Precipitate about half crystalline and half amorphous. Temp. 21° (1)	2.22 p.m., Feb. 19, 1915. Precipitate contains a very few crystals, but is still almost entirely amorphous. Temp. 21° (1)
	(1) Between Dec. 7, 1914 and Feb. 19, 1915 the temperature varied between 10° and 25°	(1) Between Dec. 7, 1914 and Feb. 19, 1915 the temperature varied between 10° and 25°

EXPLANATION OF EXPERIMENTS 6 TO 12 INCLUSIVE

These experiments were carried out on membranes of $Cu_2Fe(CN)_6$, $Ca_3(PO_4)_2$ and magnesium phosphate (see p. 460) precipitated on the inner surfaces of such porous cups as are described on p. 462. The dimensions and capacity of the cups are given on p. 462. The preliminary treatment of the cups and the general method by which the membranes were precipitated are given on pp. 461 to 463. The succeeding protocols give a condensed history of the behavior of the osmotic cells from the period when the actual membrane formation was begun.

The solutions described are what Morse calls "weight-normal solutions" (Osmotic pressure of aqueous solutions, Chapter V). That is, a "molecular" solution of cane sugar would mean a solution made by adding 342 grams of cane sugar to a litre of water. In the same way,

a "0.5M NaCl + 0.5M KCl solution" would mean a solution made by adding 29.2 grams of NaCl and 37.2 grams of KCl to a litre of water.

The amounts of dissolved substances which passed through the walls of the cells were determined by using solutions of known strength at the beginning of the experiments, and making a chemical analysis of either the inner or the outer solution at the end. Which of these procedures was adopted in each particular case is shown in the individual protocols. In some cases both inner and outer solutions were analyzed; and it was then found, as was to have been expected, that more solute disappeared from the inner solution than could be recovered in the outer solution. In experiment 12, for instance, at the end of the period between January 28 and February 3, 1915, it was found that 0.18 gram NaCl had disappeared from the inner solution while only 0.04 gram was recovered in the outer solution. The 0.14 gram left unaccounted for was no doubt held in the membrane itself and in the pores of the earthenware cup.[37] The difference observed in this case was no doubt extreme. It would be much less, for instance, in experiment 6, March 16 to 20, where the membrane and pores of the earthenware cup already contained much NaCl when the experimental period in question was begun.

Sugar was determined by means of the saccharimeter. Under the conditions of the experiment this method made possible the determination in the outer solution of about 1 per cent of the sugar originally contained in the inner solution. Alcohol was determined by means of the pyknometer. The method adopted made possible the determination of the escape of about 0.5 per cent of alcohol from the inner solution. Sodium and potassium chloride were determined by the Vollhard-Arnold method of chlorine determination (see Hawk: Practical Physiological Chemistry, 3d edition, 1910, pp. 390–391); and potassium hydroxide, by titrating against HCl with azo-litmin as an indicator. These determinations are decidedly more accurate than either the sugar or alcohol determinations.

Most of the experiments were carried out at room temperature, which underwent considerable variations; and it was noted, of course, that changes in temperature caused changes in the position of the meniscus in the outlet tube independent of the osmotic changes of the quantity of fluid in the cell. The temperature changes, however, were small in comparison to the osmotic changes. Where small osmotic changes are recorded throughout the experiments, it may be taken for granted that the readings on which they depend were taken at the same temperatures.

[37] Compare Morse: Osmotic pressure of aqueous solutions, p. 213.

Experiment 6. February 3, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Feb. 5 to 6, 1914	0.05M $K_4Fe(CN)_6$	0.05M $CuSO_4$	Not determined	0	0
Feb. 6 to 9	0.05M $K_4Fe(CN)_6$	0.05M $CuSO_4$	Not determined	0	65
Feb. 9 to 17	0.05M $K_4Fe(CN)_6$	0.012M $CuSO_4$	0.5	Traces	80
Feb. 17 to 23	0.08M $K_4Fe(CN)_6$	0.012M $CuSO_4$	1.0	0	80
Feb. 23 to 26	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.22M $C_{12}H_{22}O_{11}$+ 0.007M $CuSO_4$	0.07	Not determined	50
Feb. 26 to Mar. 3	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	2.25	Not determined	65
Mar. 3 to 10	0.22M $C_{12}H_{22}O_{11}$+ 0.003 M $K_4Fe(CN)_6$	0.007M $CuSO_4$	4.7	0	130
Mar. 10 to 12	0.12M NaCl+ 0.003M $Na_4Fe(CN)$	0.12M NaCl+ 0.007M $CuSO_4$	−0.7[2]	Not determined	60
Mar. 12 to 16	0.12M NaCl+ 0.003M $Na_4Fe(CN)_6$	0.007M $CuSO_4$	5.2	Not determined	90
Mar. 16 to 20	0.12M NaCl+ 0.003M $Na_4Fe(CN)_6$	0.007M $CuSO_4$	5.8	14% of NaCl	130
Mar. 20 to 24	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	3.3	0	90

This experiment was carried out at room temperature which varied between 12° and 26°.

[1] The quantities of solute which escaped, were, throughout this experiment, determined by analyzing the outer solutions.

[2] 0.7 cc. fluid escaped from within the cell to the outer solution, as indicated by the minus sign.

Experiment 7. February 14, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Feb. 14 to 16, 1914	0.08M $K_4Fe(CN)_6$	0.22M $CuSO_4$	Not determined	0	3
Feb. 16 to 19	0.08M $K_4Fe(CN)_6$	0.22M $CuSO_4$	5.6	0	90
Feb. 19 to 20	0.08M $K_4Fe(CN)_6$	0.25M $CuSO_4$	0.17	0	65
Feb. 20 to 21	0.08M $K_4Fe(CN)_6$	0.05M $CuSO_4$	3.6	0	95
Feb. 21 to 23	0.05M $K_4Fe(CN)_6$	0.05M $CuSO_4$	Not determined	0	0
Feb. 23 to 26	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.22M $C_{12}H_{22}O_{11}$+ 0.007M $CuSO_4$	−0.7[2]	0	40
Feb. 26 to Mar. 2	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	18.6	Not determined	120
Mar. 2 to 6	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	18.2	2.7% of $C_{12}H_{22}O_{11}$	120
Mar. 6 to 10	0.12M NaCl+ 0.003M $Na_4Fe(CN)_6$	0.007M $CuSO_4$	5.46	26.6% of NaCl	90
Mar. 10 to 14	0.22M $C_{12}H_{22}O_{11}$+ 0.003M $K_4Fe(CN)_6$	0.007M $CuSO_4$	17.0	0	100

This experiment was carried out at room temperature, which varied between 12° and 26°.

[1] The quantities of solute which escaped were, throughout this experiment, determined by analyzing the outer solutions.

[2] 0.7 cc. fluid passed from within the cell to the outer solution, as indicated by the minus sign.

Experiment 8. March 9, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Mar. 9 to 11	0.075M K_2HPO_4+ 0.075M KOH	0.09M $CaCl_2$	Not determined	0	0
Mar. 11 to 13	0.075M K_2HPO_4+ 0.075M KOH	0.09M $CaCl_2$	−1.15[2]	0	53
Mar. 13 to 17	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4 0.0075M KOH	0.009M $CaCl_2$	3.9	0	100
Mar. 17 to 20	0.127M KOH+ 0.0075M K_2HPO_4	0.009M $CaCl_2$	0.1[3]	20% of KOH	60
Mar. 20 to 24	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4+ 0.0075M KOH	0.009M $CaCl_2$	1.36[4]	0	65
Mar. 24 to 28	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4+ 0.0075M KOH	0.009M $CaCl_2$	1.29	0	85

This experiment was carried out at room temperature, which varied between 15° and 26°.

[1] The quantities of solute which escaped were, throughout this experiment, determined by analyzing the outer solutions.

[2] 1.15 cc. of fluid passed from within the cell to the outer solution as indicated by the minus sign.

[3] As a result of filling the cell with alkali, there was at first a quite rapid though short-lasting passage of fluid from without inward; 0.08 cc. passed in in the first half hour and 0.04 cc. in the second half hour. In the succeeding four days, however, there was a very slight loss of fluid from the cell.

[4] The cell showed osmotic activity *immediately* after the alkali was replaced by the sugar solution. 0.15 cc. of fluid passed in in the first hour; 0.42 cc. in the first 21 hours.

Experiment 9. March 11, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Mar. 11 to 13	0.075M K_2HPO_4+ 0.075M KOH	0.09M $CaCl_2$	Not determined	0	0
Mar. 13 to 16	0.075M K_2HPO_4+ 0.075M KOH	0.09M $CaCl_2$	−1.12[2]	0	53
Mar. 16 to 21	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4+ 0.0075M KOH	0.009M $CaCl_2$	1.6	0	75
Mar. 21 to 25	0.086M KCl+ 0.032M KOH+ 0.0075M K_2HPO_4	0.009M $CaCl_2$	0.84[3]	20% of KOH	62
Mar. 25 to 28	0.22M $C_{12}H_{22}O_{11}$+ 0.0075M K_2HPO_4+ 0.0075M KOH	0.009M $CaCl_2$	0.9[4]	0	62

This experiment was carried out at room temperature, which varied between 15° and 26°.

[1] The quantities of solute which escaped, were, throughout this experiment, determined by analyzing the outer solutions.

[2] 1.12 cc. of fluid passed from within the cell to the outer solution as indicated by the minus sign.

[3] As a result of filling the cell with the alkaline solution, there was at first, in this case as in Experiment 8, an unusually rapid passage of fluid from without inward; 0.2 cc. of fluid passed in in the first hour and 0.07 cc. in the second hour.

[4] The cell showed osmotic activity in this case as in Experiment 8 immediately after the alkaline solution was replaced by the sugar solution.

Experiment 10. May 4, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CENTIMETRES OF WATER
May 4 to 6	0.2M $MgCl_2$	0.134M K_2HPO_4+ 0.092M KOH	Not determined	0
May 6 to 8	0.2M $MgCl_2$	0.134M K_2HPO_4+ 0.092M KOH	−1.12[1]	40
May 8 to June 8	0.2M $MgCl_2$	0.0134M K_2HPO_4+ 0.0092M KOH	10.3	75
June 8 to 29	0.2M $MgCl_2$	0.0134M K_2HPO_4+ 0.0092M KHO	12.0	100

This experiment was carried out at room temperature which varied between 18° and 29°. The passage of dissolved substances through the walls of the cell was not followed at all.

[1] 1.12 cc. fluid passed from within the cell to the outer solution, as indicated by the minus sign.

Experiment 11. October 29, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED[1]	PERCENTAGE OF SOLUTE WHICH ENTERED CELL FROM OUTER SOLUTION	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Oct. 29 to 30, 1914	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.095M KOH	Not determined	0	Not determined	4
Oct. 30 to Nov. 2	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.095M KOH	Not determined	0	Not determined	70
Nov. 2 to 3	0.3M $MgCl_2$	0.0134M K_2HPO_4+ 0.0095M KOH	0.33	0	Not determined	82
Nov. 3 to 7	0.12M NaCl+ 0.015M $MgCl_2$	0.0067M K_2HPO_4+ 0.0048M KOH	1.5	8.4% of NaCl	Not determined	74
Nov. 7 to 11	0.22M $C_{12}H_{22}O_{11}$+ 0.015M $MgCl_2$	0.0067M K_2HPO_4+ 0.0048M KOH	1.3	0	Not determined	64
Nov. 11 to 16	Ringer's solution[2]	0.075M K_2HPO_4	0.12	0	Not determined	52
Nov. 16 to 20	Ringer's solution[2]	0.0075M K_2HPO_4	0.6	11% of NaCl	Not determined	54
Nov. 20 to 21	Ringer's solution[2]	0.075M K_2HPO_4	−0.6[3]	Not determined	Not determined	54
Nov. 21 to 23	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.096M KOH	0	Not determined	Not determined	59
Nov. 23 to 25	0.3M $MgCl_2$	0.0134M K_2HPO_4+ 0.0096M KOH	1.14	Not determined	Not determined	64
Nov. 25 to 27	0.12M NaCl+ 0.015M $MgCl_2$	0.075M K_2HPO_4	0.33	0.5% of NaCl	Not determined	63
Nov. 27 to 30	0.114M KCL+ 0.015M $MgCl_2$	0.075M K_2HPO_4	0.27	1.3% of KCl	Not determined	58

Nov. 30 to Dec. 3	0.12M NaCl+ 0.015M $MgCl_2$	0.075M K_2HPO_4	−0.22[3]	2.4% of NaCl	Not determined	65
Dec. 3, 1914 to Feb. 12, 1915	0.12M NaCl+ 0.015M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.32	6.6% of NaCl	3.4% of KOH	66
Feb. 12 to 15	0.12M NaCl+ 0.02M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0	0	0.6% of KOH	58
Feb. 15 to 18	0.12M NaCl+ 0.02M $MgCl_2$	0.067M K_2HPO_4+ 0.048M NH_4OH	0	Not determined	Not determined	53
Feb. 18 to Mar. 18	0.12M NaCl+ 0.02M $MgCl_2$	0.067M K_2HPO_4+ 0.048M NH_4OH	0.58	Not determined	Not determined	52
Mar. 18 to 31	0.5M $C_{12}H_{22}O_{11}$	Distilled water	19.00	10.2% of $C_{12}H_{22}O_{11}$	—	90

The experiment was carried out at room temperature, which varied between 15° and 25°.

At the end of this experiment 3.7 per cent HCl was allowed to seep through the porous cup for about 10 days under a pressure of about 20 cm. of water. It was collected, analyzed, and found to contain no Ca and 0.039 gram Mg.

[1] The quantities of solute which escaped were, throughout this experiment, determined by analyzing the outer solutions.

[2] Made by adding 0.65 gram NaCl, 0.02 gram KCl, 0.025 gram $CaCl_2$, and 0.02 gram $NaHCO_3$ to 100 cc. water.

[3] Fluid passed from within the cell to the outer solution as indicated by the minus sign.

Experiment 12. December 18, 1914

TIME	INNER SOLUTION	OUTER SOLUTION	FLUID INFLOW IN CC.	PERCENTAGE OF SOLUTE ORIGINALLY CONTAINED IN CELL, WHICH ESCAPED	PERCENTAGE OF SOLUTE WHICH ENTERED CELL FROM OUTER SOLUTION	AVERAGE PRESSURE IN INTERIOR OF CELL GIVEN IN CM. OF WATER
Dec. 18 to 21, 1914	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.096M KOH	Not determined	0	Not determined	3
Dec. 21 to 29	0.3M $MgCl_2$	0.134M K_2HPO_4+ 0.096M KOH	0.86	0	Not determined	70
Dec. 29, 1914 to Jan. 4, 1915	0.3M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	1.12	0	Not determined	85
Jan. 4 to 6	0.08M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.08	3.5% of NaCl[1]	Not determined	58
Jan. 6 to 9	0.08M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.08	3.8% of NaCl[1]	0	59
Jan. 9 to 11	0.08M KCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.06	2.9% of KCl[1]	Not determined	59
Jan. 11 to 14	0.08M KCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.06	3.8% of KCl[1]	0	60
Jan. 14 to 16	0.08M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	−0.02[3]	2.1% of NaCl[1]	Not determined	60
Jan. 16 to 19	0.08M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0	3.6% of NaCl[1]	0	60
Jan. 19 to 22	0.3M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.2	0	0	65
Jan. 22 to 28	0.915M C_2H_5OH+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.54	23.5% of C_2H_5OH[2]	Not determined	65

Jan. 28 to Feb. 3	0.6M NaCl+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.58	9.7% of NaCl[2]	Not determined	72
Feb. 3 to 9	1.1M C_2H_5OH+ 0.1M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	0.38	26.7% of C_2H_5OH[2]	Not determined	64
Feb. 9 to Mar. 18	0.3M $MgCl_2$	0.067M K_2HPO_4+ 0.048M KOH	1.08	0	Not determined	72
Mar. 18 to Apr. 7	0.5M $C_{12}H_{22}O_{11}$	Distilled water	1.2	0	—	72
Apr. 7 to June 4	0.5M $C_{12}H_{22}O_{11}$	Distilled water	3.24	0	—	100

Between 11.30 a.m., Dec. 18 and 3.30 p. m., Dec. 23, 1914 the cell used in this experiment was kept at temperatures between 29° and 35°. At 9.30 a. m., Dec. 24 the temperature had fallen to 22.5°. Between that time and 9.30 a.m. Dec. 28 it varied between 22° and 36°, after which it was kept between 28° and 33° until Feb. 25, 1915. Between this date and June 4, 1915 the cell was kept at room temperature, which varied between 14° and 26°.

[1] Quantity of solute which escaped determined by analyzing the outer solution.

[2] Quantity of solute which escaped determined by analyzing the inner solution.

[3] 0.02 cc. fluid passed from within the cup to the outer solution, as indicated by the minus sign.

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